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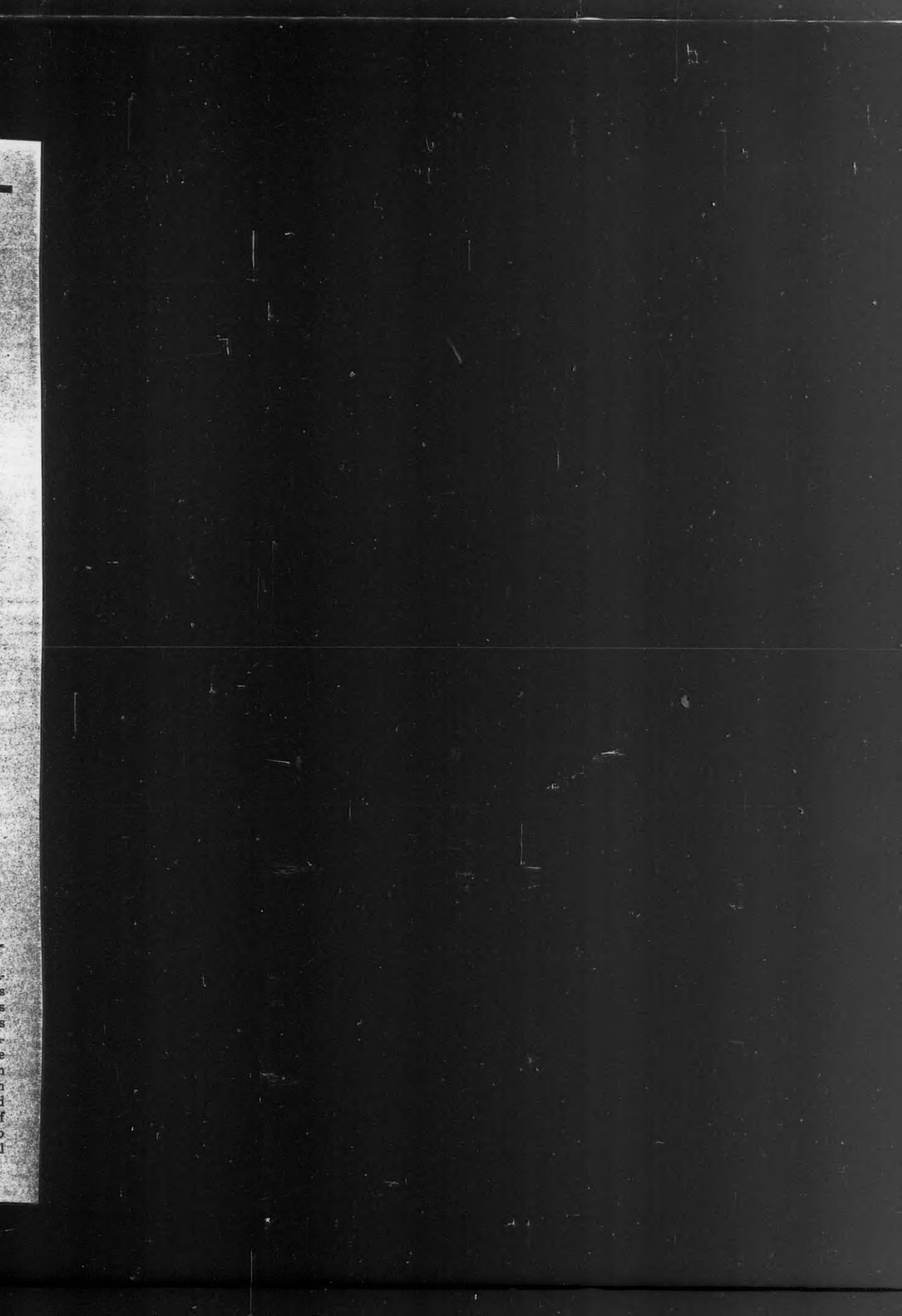

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The abundance of cetaceans in California waters. Part I: Ship surveys in summer and fall of 1991

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Abstract.—A ship survey was conducted in summer and fall of 1991 to estimate the abundance of cetaceans in California waters between the coast and approximately 555 km (300 nmi) offshore. Line-transect methods were used from a 53-m research vessel. Approximately 10,100 km were searched, and 515 groups of cetaceans were seen. The estimated abundances and coefficients of variation (in parentheses) of the most common small cetaceans are the following: 226,000 (0.28) short-beaked common dolphins, *Delphinus delphis*; 78,400 (0.35) Dall's porpoises, *Phocoenoides dalli*; 19,000 (0.41) striped dolphins, *Stenella coeruleoalba*; 12,300 (0.54) Pacific white-sided dolphins, *Lagenorhynchus obliquidens*; 9,470 (0.68) long-beaked common dolphins, *Delphinus capensis*; and 9,340 (0.57) northern right whale dolphins, *Lissodelphis borealis*. The estimated abundances (and CV's) of the most common large cetaceans are 2,250 (0.38) blue whales, *Balaenoptera musculus*; 935 (0.63) fin whales, *Balaenoptera physalus*; 756 (0.49) sperm whales, *Physeter macrocephalus*; and 626 (0.41) humpback whales, *Megaptera novaeangliae*. Estimates are also made for other species and for higher-level taxa that could not be identified to species.

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Fishery Bulletin 93:1–14 (1995).

The abundance of cetaceans in California waters is poorly known for the majority of species found there. For small cetaceans, quantitative estimates of abundance with statistical confidence limits are available only for common dolphins, *Delphinus delphis* (Dohl et al., 1986) and for harbor porpoise, *Phocoena phocoena* (Barlow, 1988). For large cetaceans, such estimates are available for gray whales, *Eschrichtius robustus* (Reilly, 1984; Buckland et al., 1993a); humpback whales, *Megaptera novaeangliae* (Calambokidis et al., 1990a, 1993[1]), and blue whales, *Balaenoptera musculus*.[1] Estimates have been made for some of the other species (Dohl et al.[2,3]), but these estimates are more than 10 years old, and most lack information on statistical precision.

Many, and perhaps all, cetaceans in California waters are vulnerable to entanglement and death in gillnet fisheries. A program is now in place to estimate the incidental mortality of cetaceans in the California gillnet fisheries (Lennert et al., in press). It is difficult, however, to assess the impact of gillnet mortality on cetacean populations without knowing population sizes. Coordinated ship and aerial surveys were initiated recently to estimate the abundance of all cetacean species in the region of California gillnet fisheries. To evaluate the effect of seasonality on cetacean abundance, surveys were designed to cover both cold-water months (Feb–Apr) and warm-water months (Jul–Nov). A ship survey was conducted during the warm-water period of 1991; an aerial survey was conducted during the cold-water periods of both 1991 and 1992. Results from the ship survey are reported here; population estimates from the aerial surveys are reported in a companion paper (Forney et al., this issue).

Field methods

A line-transect survey was conducted from 28 July to 5 November 1991 with the 53-m National Oceanographic and Atmospheric Admin-

[1] Calambokidis, J., G. H. Steiger, and J. R. Evenson. 1993. Photographic identification and abundance estimates of humpback and blue whales off California in 1991–92. Final Contract Rep. 50ABNF100137, submitted to the Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038, 40 p.

[2] Dohl, T. P., K. S. Norris, R. C. Guess, J. D. Bryant, and M. W. Honig. 1978. Cetacea of the Southern California Bight. Part II of Summary of marine mammals and seabird surveys of the Southern California Bight area, 1975–78. Final Rep. to the Bureau of Land Management, 414 p. [NTIS Rep. No. PB81248189.]

[3] Dohl, T. P., R. C. Guess, M. L. Duman, and R. C. Helm. 1983. Cetaceans of central and northern California, 1980–83: status, abundance, and distribution. Final report to the Minerals Management Serv., Contract No. 14-12-0001-29090, 284 p.

istration (NOAA) vessel *McArthur* to assess the abundance of cetaceans in California waters. Primary cruise tracks were drawn for a unifirm survey of the 814,900 km^2 area between the 18-m (10-fathom) isobath and approximately 555 km (300 nmi) offshore (Fig. 1).

Primary observation team

The basic survey method was that which was developed and used to estimate the abundance of small cetaceans in the eastern tropical Pacific (Holt and Powers, 1982; Holt, 1987; Holt and Sexton, 1989; Wade and Gerrodette, 1993). The primary observation team consisted of three observers who searched from a viewing height of 10 m above the sea surface: two observers searched with 25× pedestal-mounted binoculars; the third observer searched with unaided eye, and (occasionally) 7× binoculars, and also served as data recorder. Observers rotated among these three duty stations every 1/2 hour, and two observer teams alternated work and rest periods every two hours. Sighting effort was maintained from dawn to dusk whenever weather conditions allowed, and searching covered the entire region from directly in front of the vessel to 90 degrees left and right and out to the horizon. Data were recorded on a lap-top computer that had direct input from the ship's GPS (Global Positioning System) navigation system. Recorded data included sighting conditions (sea state, cloud cover, sun position, etc.), observer positions, the beginning and end of effort, and information pertaining to sightings.

When a sighting was made, all observers were made aware of the animals' location. The perpendicular distance from the trackline to the center of the group was estimated from the initial bearing and distance. The initial bearing of a cue (a blow, a splash, or a sighting of animals) was measured relative to the bow of the vessel by means of a calibrated collar on the base of the yoke of the 25× binoculars. The initial distance was typically estimated from a calibrated reticle scale in the oculars of both the 25× and 7× binoculars with the formula derived by Smith (1982) and was calibrated by using radar-measured distances to inanimate objects (Barlow and Lee, 1994). If a shore horizon was closer than 11.1 km (6 nmi), distance was estimated by comparison with the radar-measured distance to shore. Occasionally, for very close animals seen only by the third observer, sighting distances and angles were estimated by eye. If a cue turned out to be a cetacean, effort was interrupted and the ship was typically diverted towards the animals in order to obtain estimates of species composition and group size. The vessel was not typically diverted for cetaceans that were greater than 5.55 km (3 nmi) perpendicular distance from the trackline.

Species identification was made collectively by the team, but quantitative estimates of species composition and group size were made independently by each observer. For estimation purposes, a group was defined as a collection of closely associated individuals (typically within several body lengths of each other) that exhibited cohesive behavior. In the field, however, a single distant sighting might prove to be two behaviorally distinct groups upon closer inspection. In such cases, when it was impossible to determine which was the original group sighted, both groups were pooled to estimate group size and species composition. For mixed-species groups, species composition was recorded as an observer's estimate of the percentage of each species present in the group. The observers recorded species composition and group-size data in confidential personal notebooks, and the data were transcribed at the end of the day into the computer data record by the cruise leader.

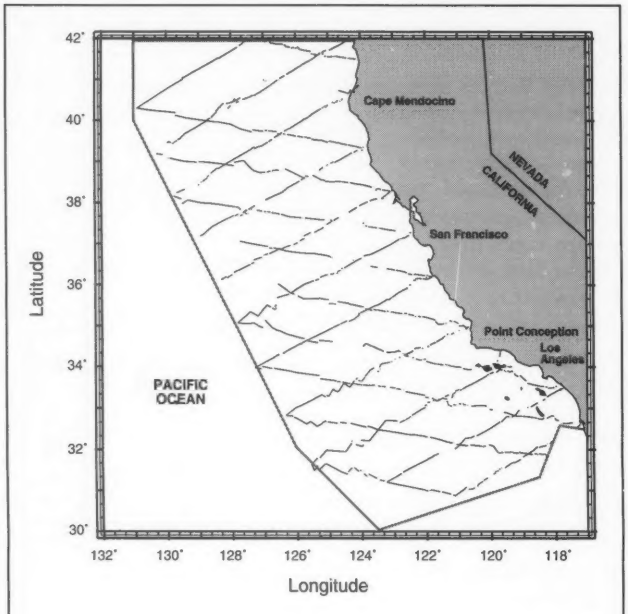

Figure 1

Transect lines (thin solid lines) completed during the survey. The bold polygon indicates the limit of the main study area.

Species identification

Observers attempted to classify all the species present in a group to the lowest possible taxonomic level (one member of each team was a cetacean identification expert with at least nine months of at-sea survey experience on prior marine mammal surveys). Several higher taxonomic groups were used in cases where species identification was not possible. These higher groups were beaked whales of the genus *Mesoplodon*; unidentified sei or Bryde's whales; unidentified beaked whales (including members of the genera *Mesoplodon* and *Ziphius*); unidentified large whales (including members of the species group "large whale" in Table 1 as well as the genera *Eschrichtius* and *Eubalaena*); unidentified baleen whales (including members of the genera *Balaenoptera*, *Megaptera*, *Eschrichtius*, and *Eubalaena*); unidentified small whales (including members of the species groups "small whales" and "large delphinids" in Table 1); unidentified delphinoids (including members of the species groups "small delphinids," "large delphinids," and "cryptic species" in Table 1); and unidentified cetaceans (which could include any of the species listed above or in Table 1). The number of sightings identified to these higher taxonomic levels is relatively small, and these animals were not included in the abundance estimates for individual species.

Conditionally independent observer

In addition to the primary observation team, a fourth observer was on duty 81% of the time and looked for cetaceans that were missed by the primary team. This conditionally independent observer was stationed immediately next to the other observers, searched with 7× binoculars and unaided eyes, and did not reveal the presence of cetaceans until after they were clearly missed by the primary observation team (i.e. after they had passed abeam of the vessel or were bow-riding). Nine different people served as independent observers during the survey, and all worked irregular schedules that overlapped with both primary teams. Independent observers did not work more than two consecutive hours. When a sighting was made by the independent observer, that person maintained their normal behavior so as to avoid drawing the attention of the primary observer team. Initial bearing and distance were estimated by eye or with the aid of reticles in the ocular of 7× binoculars and a hand-held protractor. After a group was clearly missed by the primary team, the independent observer announced the presence of the animals to the data recorder and gave the initial bearing and distance. Typically the vessel was diverted towards the group, and species composition and group size were estimated by the primary observation team.

Analytical methods

Cetacean abundance was estimated from survey data with line-transect methods (Buckland et al.,

Table 1

Number of groups of cetaceans which contained members of the indicated species and species groups. The sum of all species in a group may be greater than the total for that group because the latter contains mixed-species groups. Totals do not include off-effort sightings.

Species group and species	No. of sightings
Small delphinids	285
short-beaked common dolphin, *Delphinus delphis*	123
long-beaked common dolphin, *Delphinus capensis*	6
unclassified common dolphin, *Delphinus* spp.	8
striped dolphin, *Stenella coeruleoalba*	24
Pacific white-sided dolphin, *Lagenorhynchus obliquidens*	12
northern right whale dolphin, *Lissodelphis borealis*	16
unidentified delphinoid	21
Cryptic species	132
harbor porpoise, *Phocoena phocoena*	32
Dall's porpoise, *Phocoenoides dalli*	97
pygmy sperm whale, *Kogia breviceps*	3
Large delphinids	37
bottlenose dolphin, *Tursiops truncatus*	16
Risso's dolphin, *Grampus griseus*	29
killer whale, *Orcinus orca*	5
Large whales	127
sperm whale, *Physeter macrocephalus*	13
Baird's beaked whale, *Berardius bairdii*	1
Bryde's whale, *Balaenoptera edeni*	1
Bryde's or sei whale, *Balaenoptera edeni* or *B. borealis*	2
fin whale, *Balaenoptera physalus*	22
blue whale, *Balaenoptera musculus*	49
humpback whale, *Megaptera novaeangliae*	13
unidentified baleen whale	9
unidentified large whale	22
Small whales	48
unidentified beaked whale	7
mesoplodont beaked whale (*Mesoplodon* spp.)	5
Cuvier's beaked whale, *Ziphius cavirostris*	14
minke whale, *Balaenoptera acutorostrata*	4
unidentified small whale	11
unidentified cetacean	8

1993b). The basic equation for estimating abundance, N, for grouped animals with line transect is given by

$$N = \frac{AnSf(0)}{2Lg(0)}, \quad (1)$$

where A = size of the study area;
n = number of sightings;
S = mean group size;
$f(0)$ = sighting probability density at zero perpendicular distance;
L = length of transect line completed; and
$g(0)$ = probability of seeing a group directly on the trackline.

Ideally, S, $f(0)$, and $g(0)$ would be estimated separately for each species. However, the presence of mixed-species groups and small sample sizes required pooling for the estimation of $f(0)$ and $g(0)$. The parameter $f(0)$ was estimated with the Hazard rate model (Buckland, 1985). This model was fitted by maximum likelihood with ungrouped perpendicular distances. Perpendicular distances were estimated from bearing and radial distance estimates made by observers.

Pooling and stratification for estimating *f* (0)

Pooled $f(0)$'s were estimated for five species groups: "small delphinids," "large delphinids," "small whales," "large whales," and "cryptic species." The five species groups were defined to include all of the species seen on the survey (Table 1) and were based on patterns of species cooccurrence in groups and on similarities in the physical and behavioral attributes that affect sightability from a ship. As an example, bottlenose dolphins, *Tursiops truncatus*, were never seen in a single-species group but were seen with Risso's dolphins, *Grampus griseus*, 13 times, with striped dolphins, *Stenella coeruleoalba*, one time, and with sperm whales, *Physeter macrocephalus*, three times. Bottlenose dolphins were pooled together with Risso's dolphins because they were seen most frequently with that species and because their sighting characteristics are more similar to Risso's dolphins (medium body size, prominent dorsal fin, occasional low puffy blow, small to medium group size) than to the other two species with which they were seen. Because killer whales, *Orcinus orca*, were never seen with other species but share the same sighting characteristics, these were also included in the species group "large delphinids." The other four groups are "small delphinids" which are of small body size (2–3 m) and are found in medium to large groups; "small whales" which are of medium body size (4–10 m), typically show no blow, often surface inconspicuously, and are typically found in small groups; "large whales" which are of large body size (10–30 m), almost always show a conspicuous blow, and are found in small to medium groups; and "cryptic species" which are small (1.5–4.0 m), show no blow, typically surface inconspicuously, and are found in small groups. The assignment of higher-than-species taxa to species groups is given in Table 1.

In estimating $f(0)$ for each species group, I explored stratification by two factors that are likely to affect sightability: sea state and group size. To avoid estimating more parameters than are justified by the data, I chose the most parsimonious stratification model by minimizing Akaike's Information Criterion (AIC) (Akaike, 1973), defined as 2 multiplied by the number of parameters used to estimate $f(0)$ *minus* 2 multiplied by the sum of the log-likelihoods of the fitted values of $f(0)$. Sea state was subjectively stratified into calm (Beaufort 0–2) and rough (Beaufort 3–5), based on the obvious degradation in sighting conditions that occurs with the presence of whitecaps at Beaufort 3. I stratified by group size by first finding the group size that divided the data into two samples with approximately the same number of sightings in each. If this stratification resulted in a lower AIC, I explored further stratification into three samples of approximately equal size.

The above approach to stratification resulted in different strata for each species group. For small delphinids, AIC was minimized by stratifying group size into the categories 1–20, 21–100, and >100. For large delphinids, optimal stratification was with group size categories of 1–20 and >20. For large whales, AIC was minimized by using group size strata of 1–3 and >3. Because "cryptic species" and "small whales" were seldom seen in rough conditions, I estimated abundance for these species by using only data from calm conditions and did not explore stratification by sea state. Group size stratification resulted in higher AIC values for "cryptic species" and "small whales," so these groups were not stratified by group size. Sea-state stratification was not chosen on the basis of AIC values for any species group.

In stratification by group size, estimates of density in the various strata are added together to give an overall density. The equation for estimating abundance of each species k is therefore given by

$$N_k = \sum_{j=1}^{3} \frac{An_{j,k}S_{j,k}f_{j,k}(0)}{2Lg_{j,k}(0)}, \quad (2)$$

where A = size of study area;

$n_{j,k}$ = number of sightings of species k in group size stratum j;
$S_{j,k}$ = mean group size of species k in group size stratum j;
$f_{j,k}(0)$ = sighting probability density at zero perpendicular distance for group size stratum j of the species group to which species k belongs;
L = length of transect line completed; and
$g_{j,k}(0)$ = probability of detecting a group directly on the trackline for group size stratum j of the species group to which species k belongs.

Perpendicular distance truncation

Sightings of distant groups add little to the estimation of trackline density and can introduce bias. Buckland et al. (1993b) recommend truncating to eliminate at least the most distant 5% of all sightings. In the current study, groups of cetaceans were typically not pursued for species identification and group size estimation if they were farther than 5.5 km (3 nmi) from the trackline. Therefore, by survey design, perpendicular distances must be truncated at no more than 5.5 km. I used a truncation distance of 3.7 km (2 nmi) for "small delphinids," "cryptic species," "large delphinids," and "small whales," which eliminated 8.8%, 2.4%, 4.6%, and 12.8% of all groups (respectively). A truncation distance of 5.5 km was used for "large whales," which eliminated 10.9% of groups.

Group-size estimation

The estimation of group size for cetaceans is difficult and can lead to bias in the estimation of abundance. To avoid bias, correction factors were developed for individual observers. The estimates of four of the six primary observers on the present survey had been previously calibrated by means of aerial photographic estimates to represent "true" group size.[4] The "best" estimates of two of these four were found to indicate group size with accuracy and did not require any correction factors. The other two required correction factors, and, for one, correction factors varied significantly from one year to the next. A helicopter was not available to make aerial photographic estimates of group size on the present survey, so correction factors for individual observers were estimated indirectly by comparison with the two observers who, in the previous study, did not require correction.

Linear regression was used to compare one observer's estimates of group size to another's for the subset of groups that were estimated by both. Group sizes were $\log_{10}$-transformed to normalize variances. For the two observers who did not require a correction factor in the previous study,[4] the slope of the regression was 1.009 (SE=0.017), indicating that, relative to each other, the observers were still estimating group size consistently. Correction factors for the other four observers were based on the slope and intercept of the regression of their "best" estimates against the mean of "best" estimates of the two who did not need calibration.

The group size for each species in a group was estimated as the average of all observers' corrected estimates of the size of the group multiplied by the average of all observers' estimates of the percentage of that species present (if in a mixed-species group).

Probability of detecting trackline groups

Estimating the probability that a group on the transect line will be seen, $g(0)$, is fraught with difficulties (see Buckland et al. [1993b] for a review of previous attempts). In the context of bias from missed groups of marine mammals, it is useful to think in terms of the dichotomy proposed by Marsh and Sinclair (1989): bias can result from groups that were available to be seen but were not (perception bias) and from groups that were not available to be seen either because they did not surface or because they surfaced behind a swell (availability bias). I will make a minimum estimate of perception bias based on data collected by the conditionally independent observer and on the approach given in the Appendix. Because the sample of sightings made by independent observers is small (only 37 cetacean groups), $f_2(0)$ in Equation 7 was estimated for all cetaceans pooled without stratification by group size or sea state. Perpendicular distance data were fitted with the Hazard rate model to estimate $f_2(0)$. (Groups are only available to the independent observer if they were missed by the other observers; therefore the distribution of perpendicular distances need not be monotonically decreasing. In this case, however, it was, and a more general model is not likely to have performed better than the Hazard rate model.) The analytical variances of $f_1(0)$ and $f_2(0)$ (from the information matrix method) were used in estimating the coefficient of variation of $g_1(0)$ from Equation 8, and the variances of n_1 and n_2 were estimated by assuming a Poisson distribution. Consideration of availability bias is deferred to the Discussion section.

[4] Gerrodette, T. D., and C. Perrin. 1991. Calibration of shipboard estimates of dolphin school size from aerial photographs. Admin. Rep. LJ-91-36, available from Southwest Fish. Sci. Cent., P.O. 271, La Jolla, CA 92038, 73 p.

Coefficients of variation and confidence intervals

Coefficients of variation (CV) and confidence intervals (CI) of the abundance estimates are based on the bootstrap method (Efron, 1977; Buckland et al., 1993b). The sightings associated with consecutive segments of search effort were combined to form a set of subsamples of 139 km (75 nmi) of search effort (corresponding to approximately one day of survey effort).[5] I drew subsamples randomly with replacement from this set of effort segments, and a pseudo-population size was estimated by using the same group size stratification as was used for the actual abundance estimates. For each bootstrap sample, the probability of detecting trackline groups, $g(0)$, was estimated as a random number between 0 and 1 drawn from the probability distribution of a binomial ratio with a mean and coefficient of variation equal to the estimated values. This process was repeated 1,000 times, and the CV of the estimated population size was calculated as the standard error of the 1,000 pseudo-population sizes divided by the estimated population size. Bootstrap 95% confidence intervals on the population estimates were based on the 25th and 976th ranked estimates from the bootstrap samples. Log-normal 95% confidence intervals were based on the method given by Buckland et al. (1993b) and used the bootstrap estimate of CV.

Results

During the survey approximately 10,100 km of searching effort were completed (Fig. 1), and 515 cetacean groups were seen during the sampling effort. Tracklines included 2,386 km in calm sea states (Beaufort 2 or less) and 7,696 km in rough sea states (Beaufort 3–5). During the survey, 18 cetacean species were identified (as well as at least one species that could only be identified to genus) (Table 1). More detailed data summaries for this survey are presented by Hill and Barlow (1992), including the positions and school sizes of all on- and off-effort sightings of cetaceans and pinnipeds, maps showing the distribution of sightings for each species, distributions of perpendicular distances for each species, patterns of association in mixed-species groups, summaries of searching effort completed under various conditions, and sighting rates of individual observers. The fit of the probability density functions to the distributions of perpendicular distances are illustrated by Barlow.[5]

[5] Barlow, J. 1993. The abundance of cetaceans in California waters estimated from ship surveys in summer/fall 1991. Admin. Rep. LJ-93-09, available from Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038, 39 p.

Group-size estimation

Group-size correction parameters, the slopes and intercepts (in parentheses) of $\log_{10}$-transformed regressions, were 0.922 (0.03), 1.022 (–0.03), 0.886 (0.07), and 0.777 (0.11) for the four observers who required correction. Three of these observers appear to have underestimated group size, in some cases by a large amount (a group of 500 would have been, on average, estimated as 328, 534, 283, and 152 by these four observers, respectively).

Probability of detecting trackline groups

Independent observers searched a total of 8,190 km. Approximately 7% of groups were detected only by the independent observer; however, all groups that were detected only by the independent observer were groups of less than 20 individuals and accounted for only 0.7% of the individuals that were seen on the survey. Of all groups that had less than 20 animals and were seen while the independent observer was on duty, 347 were seen by the primary observers, and 40 were seen by the independent observer.

Abundance estimation

With estimated values of $f(0)$ and $g(0)$ (Table 2), density and abundance were calculated for 19 cetacean species and 9 higher taxonomic categories (Table 3). Common dolphins were the most abundant cetaceans by a large margin. Of the two recently recognized common dolphin species (Heyning and Perrin, 1994), the short-beaked variety was much more abundant than the long-beaked variety. Blue whales were the most abundant species of large whale.

Discussion

Distribution

The distributions of cetaceans seen during this survey (Figs. 2–6) are in general agreement with the results of other studies in this area (Leatherwood et al., 1982; Dohl et al., 1986; Smith et al., 1986; Barlow, 1988; Forney et al., this issue; Dohl et al.[2,3]). However, the observed distribution of some species contradicted results of previous studies. Striped dolphins were seen rather commonly in mixed groups with short-beaked common dolphins in southern and central California between 185 and 555 km (100–300

Table 2

Estimated values of $f(0)$ and $g(0)$ for each of the species group stratifications which were chosen on the basis of Akaike's Information Criterion (AIC) minimization. Truncation distances for estimating $f(0)$ are 5.5 km for large whales and 3.7 km for all other species. Sample sizes include the total number of groups seen by the primary team, n, the number of groups seen by the primary team when an independent observer was on duty, n_1, and the number of groups seen by the independent observers but not by the primary team, n_2. NA indicates information that is not available because it could not be estimated. CV is the coefficient of variation.

Main stratum and substrata	Number of sightings			Primary observers		Secondary observers		Primary observers	
	n	n_1	n_2	$f(0)$ km^{-1}	CV $f(0)$	$f(0)$ km^{-1}	CV $f(0)$	$g(0)$	CV $g(0)$
Small delphinids (3.7 km truncation)									
group size 1–20	67	58	9	1.258	0.249	1.864	0.147	0.770	0.137
group size 21–100	58	51	0	0.944	0.336	1.864	0.147	1.000	NA
group size 101+	47	44	0	0.283	0.193	1.864	0.147	1.000	NA
Cryptic species (3.7 km truncation)									
calm seas	102	78	14	1.574	0.199	1.864	0.147	0.787	0.103
Large delphinids (3.7 km truncation)									
group size 1–20	15	14	1	0.504	0.306	1.864	0.147	0.736	0.391
group size 21+	17	17	0	0.352	NA	1.864	0.147	1.000	NA
Large whales (5.5 km truncation)									
group size 1–3	87	81	3	0.696	0.278	1.863	0.146	0.901	0.073
group size 4+	26	22	0	0.256	NA	1.863	0.146	1.000	NA
Small whales (3.7 km truncation)									
calm seas	23	19	1	0.614	0.488	1.864	0.147	0.840	0.218

nmi) from shore. Although striped dolphins were known to inhabit this area (Leatherwood et al., 1982), their frequency of occurrence was much greater than expected. Blue whales were seen primarily in southern California between 92 and 370 km (50–200 nmi) offshore. In previous years, this species was seen commonly in central California between the coast and 92 km (50 nmi) offshore (Calambokidis et al., 1990b). One species was surprising in its absence: short-finned pilot whales, *Globicephala macrorhynchus*, were previously common in southern California, especially around the Channel Islands in winter (Leatherwood et al., 1982). (Note: one group of pilot whales was seen and photographed by independent researchers between San Francisco and Monterey on 2 November 1991.[6])

Abundance

Abundance estimates from this study are also in general agreement with previous esti-

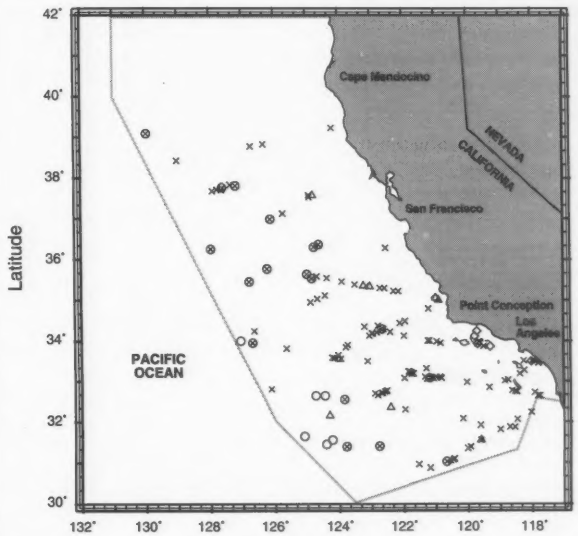

Figure 2

Locations of on-effort sightings of short-beaked common dolphins (×), long-beaked common dolphins (◇), unidentified common dolphins (△), and striped dolphins (○). Scientific names are given in Table 1.

[6] Jones, P. A., and I. D. Szczepaniak. 1992. Report on the seabird and marine mammal censuses conducted for the long-term management strategy (LTMS), August 1990 through November 1991, for the U.S. Environmental Protection Agency, Region IX, San Francisco. July 1992.

Table 3

Number of groups seen (n), mean group size (S), density of individuals, abundance estimates (N), 95% confidence intervals (CI) on those estimates, and coefficients of variation (CV) for all species and higher taxa that were identified. Density estimates are based on lengths of transect given in the text and estimates of $f(0)$ and $g(0)$ given in Table 2. Mean group size includes only the indicated species and can therefore be less than the minimum of the group size category (which is defined based on the total number of all species present). Scientific names are given in Table 1.

						Boot strap		Log-normal	
Species strata	Number of groups n	Mean group size S	Animal density km^{-2}	Pop. size N	CV	Lower 95% CI	Upper 95% CI	Lower 95% CI	Upper 95% CI
Small delphinids									
short-beaked common dolphin			3.248	225,821	0.279	143,026	419,911	132,139	385,918
group size 1–20	25	11.0	0.261						
group size 21–100	52	44.7	1.274						
group size 101+	39	267.3	1.713						
long-beaked common dolphin			0.136	9,472	0.683	0	27,029	2,817	31,842
group size 1–20	1	11.8	0.011						
group size 21–100	0	0.0	0.000						
group size 101+	4	190.2	0.125						
common dolphin (unclassified)			0.148	10,286	0.815	573	37,007	2,539	41,664
group size 1–20	6	5.4	0.031						
group size 21–100	1	15.1	0.008						
group size 101+	1	661.5	0.109						
striped dolphin			0.273	19,008	0.412	8,234	45,864	8,755	41,267
group size 1–20	2	7.7	0.015						
group size 21–100	5	29.3	0.080						
group size 101+	14	77.6	0.178						
Pacific white-sided dolphin			0.177	12,310	0.537	1,888	27,965	4,590	33,010
group size 1–20	7	11.5	0.076						
group size 21–100	3	46.2	0.076						
group size 101+	2	75.4	0.025						
northern right whale dolphin			0.134	9,342	0.567	2,125	21,488	3,322	26,272
group size 1–20	10	9.9	0.094						
group size 21–100	3	9.4	0.015						
group size 101+	2	75.7	0.025						
unidentified delphinoid			0.052	3,603	0.462	1,180	6,197	1,521	8,536
group size 1–20	17	3.2	0.052						
group size 21–100	0	0.0	0.000						
group size 101+	0	0.0	0.000						
Cryptic species									
harbor porpoise[1]	31	5.0	0.758	52,743	0.682	0	147,905	15,714	177,026
Dall's porpoise	69	3.3	1.127	78,422	0.354	33,462	150,487	40,026	153,649
pygmy sperm whale	2	1.3	0.013	870	0.796	0	2,741	220	3,433
Large delphinids									
bottlenose dolphin			0.022	1,503	0.481	499	3,819	615	3,674
group size 1–20	4	2.8	0.004						
group size 21+	10	8.3	0.017						
Risso's dolphin			0.122	8,496	0.415	4,236	21,676	3,890	18,555
group size 1–20	12	8.3	0.039						
group size 21+	16	25.2	0.082						
killer whale			0.004	307	1.196	0	2,340	48	1,947
group size 1–20	3	3.7	0.004						
group size 21+	0	0.0	0.000						
Large whales									
sperm whale			0.011	756	0.493	211	1,537	303	1,886
group size 1–3	4	1.2	0.002						
group size 4+	9	6.6	0.009						

Table 3 (Continued)

Species strata	Number of groups n	Mean group size S	Animal density km^{-2}	Pop. size N	CV	Boot strap Lower 95% CI	Boot strap Upper 95% CI	Log-normal Lower 95% CI	Log-normal Upper 95% CI
Baird's beaked whale			0.001	38	1.025	0	127	7	203
group size 1–3	0	0.0	0.000						
group size 4+	1	3.7	0.001						
Bryde's whale			0.001	61	1.078	0	242	11	339
group size 1–3	1	1.9	0.001						
group size 4+	0	0.0	0.000						
Bryde's or sei whale			0.001	63	1.093	0	232	11	355
group size 1–3	2	1.0	0.001						
group size 4+	0	0.0	0.000						
fin whale			0.013	935	0.635	130	2,607	299	2,925
group size 1–3	17	1.4	0.011						
group size 4+	4	4.7	0.003						
blue whale			0.033	2,250	0.381	899	4,131	1,093	4,632
group size 1–3	36	1.6	0.026						
group size 4+	13	3.3	0.007						
humpback whale			0.009	626	0.411	196	1,133	289	1,359
group size 1–3	7	1.8	0.006						
group size 4+	3	7.3	0.003						
unidentified baleen whale			0.003	214	0.631	26	530	69	665
group size 1–3	5	1.2	0.003						
group size 4+	1	2.1	0.001						
unidentified large whale			0.009	629	0.470	167	1,306	262	1,508
group size 1–3	15	1.3	0.009						
group size 4+	0	0.0	0.000						
Small whales									
unidentified beaked whale	3	3.5	0.019	1,322	0.892	0	4,541	295	5,921
mesoplodont beaked whale	2	1.0	0.004	250	0.834	0	746	60	1,040
Cuvier's beaked whale	7	1.9	0.023	1,621	0.823	186	5,555	396	6,637
minke whale	4	1.1	0.008	526	0.971	0	2,244	106	2,596
unidentified small whale	5	1.0	0.009	645	0.767	127	2,061	170	2,446
unidentified cetacean	3	1.7	0.009	620	0.879	0	2,026	141	2,731

[1] More precise estimates for harbor porpoise are recently available in Barlow and Forney (1994).

mates (Dohl et al., 1986; Barlow, 1988; Calambokidis et al., 1990a; Dohl et al.[2,3]). This is the first cetacean survey in California waters to include the region between 277 and 555 km (150–300 nmi) offshore. The studies of Dohl et al.[2,3] included only the inshore 185 km (100 nmi) of the present study area, making direct abundance comparisons difficult. The mark-recapture population estimates of blue and humpback whales by Calambokidis et al. (1990, a and b) were based on individuals sighted near the coast. Furthermore, the estimates of Dohl et al.[2,3] do not have associated statistical confidence intervals. Hence, accurate comparisons with previous studies can be made only for the more coastal species and meaningful statistical tests of differences can be made for even fewer species. Direct comparisons with the 1991 and 1992 aerial surveys (Forney et al., this issue) are planned for future publications.

The abundance of harbor porpoise estimated for 1984 and 1985 was approximately 9,576 (CV=0.51) (Barlow [1988] his regions 1–4), which is smaller than the present estimate of 52,700 (CV=0.68). This discrepancy may be due to the inappropriate design of the present survey for a coastal species such as harbor porpoise.

Humpback whale abundance in central California was estimated as 338 based on aerial surveys from August to November of 1980–83 (Dohl et al.[3]); however, this estimate does not include a correction factor for submerged whales. Based on mark-recapture methods, the abundance of humpback whales in 1991 and 1992 was estimated to be 581 (CV=0.03).[1] This estimate is

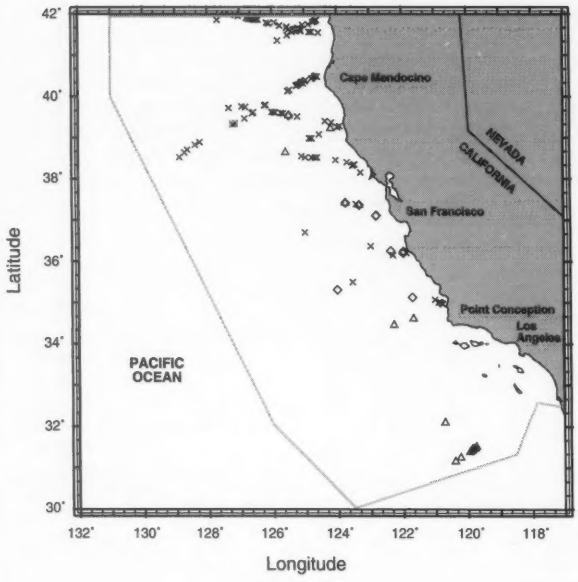

Figure 3

Locations of on-effort sightings of Dall's porpoise (×), northern right whale dolphins (△), and Pacific white-sided dolphins (◇). Scientific names are given in Table 1.

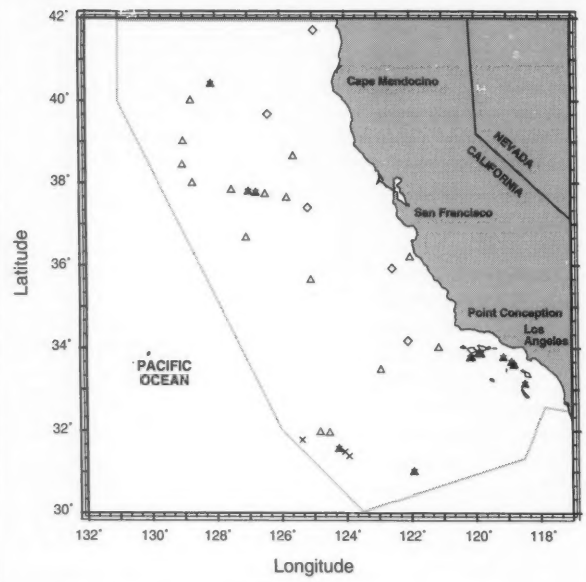

Figure 4

Locations of on-effort sightings of killer whales (◇), Risso's dolphins (△), and bottlenose dolphins (×). Scientific names are given in Table 1.

very close to the present estimate of 626 and is well within its 95% confidence interval.

For two species, new estimates of abundance appear to be substantially different from previous estimates. For the late 1970's, the combined summer and fall estimate of common dolphin abundance was 57,270 (CV=0.17) (Dohl et al., 1986). Although the methods used were very different and the area surveyed was smaller in that study, estimates for other small cetaceans are similar in the two studies. A large increase in common dolphin abundance is likely. This could have resulted as an effect of the 1991–92 El Niño. Although there were no surface temperature manifestations of El Niño in the study area at the time of the survey, it is possible that common dolphins were moving into California waters from farther south as a result of El Niño changes there. Since 1980, a decline has been noted in the abundance of the northern stock of common dolphins south of 30°N (Anganuzzi et al., 1993), and those authors hypothesize that this could have been caused by a general northward movement of that stock. This interpretation is consistent with the increases noted here, but the magnitude of the decrease in the south (from approximately 500,000 in 1980 to approximately 100,000 in 1991 [Anganuzzi et al. 1993]) is greater than the entire estimated population in California waters.

The abundance of blue whales, based on the current line-transect data (2,250), is also much higher than recent estimates made from individual-identification mark-recapture techniques (904 based on left-side photographs and 1,112 based on right-side photographs).[1] Although some mark-recapture estimates may be biased low because of geographic heterogeneity in habitat use by individual whales (Hammond, 1990), the methods used for mark-recapture should have minimized those effects.[1] South of the present study area, the abundance of blue whales was estimated to be 1,415 (CV=0.24) based on line-transect ship surveys in the eastern tropical Pacific from 1986 to 1990 (Wade and Gerrodette, 1993). The latter study included sightings made along the coast of Baja California (which probably belong to the California feeding population) as well as sightings made near the Costa Rica Dome and along the Equator (which are likely to be part of a different population; Reilly and Thayer [1990]).

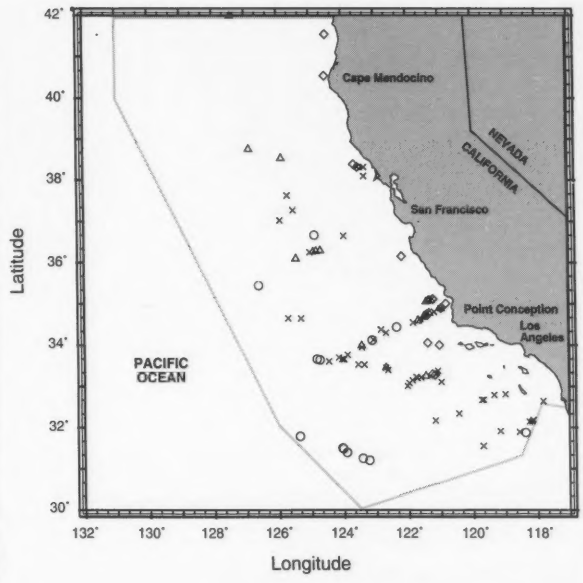

Figure 5

Locations of on-effort sightings of fin whales (△), humpback whales (◇), blue whales (×), and sperm whales (○). Scientific names are given in Table 1.

Probability of detecting trackline groups

The probability of detecting a trackline group of animals, $g(0)$, varied between 0.74 and 1.0 (Table 2). The data clearly indicated that small groups are much more likely to be missed than are large groups. This is intuitively obvious and justifies stratifying by group size when estimating $g(0)$ values. The fraction of trackline harbor porpoise seen in calm seas has been estimated previously to be 0.78 (with five observers on a similar platform in California, Barlow [1988] and 0.70 (with six observers in the Gulf of Maine, Palka [1993]). The higher value of $g(0)$ estimated here for "cryptic species" with only three observers (0.81) may be due to the inclusion of Dall's porpoise which may be easier to see or may simply be an artifact of small sample size.

These estimates of the fraction of animals seen include only animals that were available to be seen. Availability bias is likely to be large for species such as beaked whales, which have extremely long dive times, and harbor porpoise and Dall's porpoise, which have shorter dive times but seldom are seen more than 0.5 km from the ship and may therefore remain submerged during the entire time they are within visual range. Correcting for availability bias is more difficult than for perception bias. Attempts that have been made so far have involved detailed modeling of the surfacing behavior of the animal and the searching behavior of the researchers (Doi, 1971, 1974; Barlow et al., 1988; Stern, 1992; Kasamatsu and Joyce[7]). In addition, there are still problems with estimating perception bias because the methods used here assume that all animals are equally available to be seen if they surface. Heterogeneity in sightability (e.g. animals that splash vs. animals that do not) generally will result in an underestimate of the fraction missed. Additional work is needed to obtain complete estimates of the fraction of trackline animals seen for all species.

Previous studies of Dall's porpoise have shown that attraction to the vessel is a greater problem for estimating the abundance of this species than are missing trackline animals (Turnock and Boucher[8]). Turnock and Quinn (1991) estimated a correction factor of 0.2378 (CV=0.3391) to adjust Dall's porpoise abundance estimates for ship surveys (effectively then, g_0=4.2). That study was based, however, on a design that used only one observer who searched with 7× binoculars and unaided eyes. In the present study, very few Dall's porpoise appeared to be attracted to the vessel; of those sighted in calm conditions and used for abundance estimation, only 10% (9 of 88) of the Dall's porpoise groups approached the vessel to "ride the bow wave," and 89% (78 of 88) were exhibiting a "slow roll" surfacing behavior at the time they were first sighted. Because attraction to the vessel was less than in other studies and because most Dall's porpoise were sighted before showing any apparent reaction to the vessel (perhaps because 25× binoculars were used), the magnitude of bias is probably less than that estimated by Turnock and Quinn (1991).

Statistical precision

An attempt was made to account for most sources of sampling error in the bootstrap estimates of confidence intervals and coefficients of variation. However, several sources of variation could not be easily included. The process of selecting a stratification

[7] Kasamatsu, F., and G. G. Joyce. 1991. Abundance of beaked whales in the Antarctic. Int. Whaling Comm. working paper SC/43/O12.

[8] Turnock, B. J., and G. C. Boucher. 1990. Population abundance of Dall's porpoise, *Phocoenoides dalli*, in the western North Pacific Ocean. Int. Whaling Comm. working paper SC/42/SM10.

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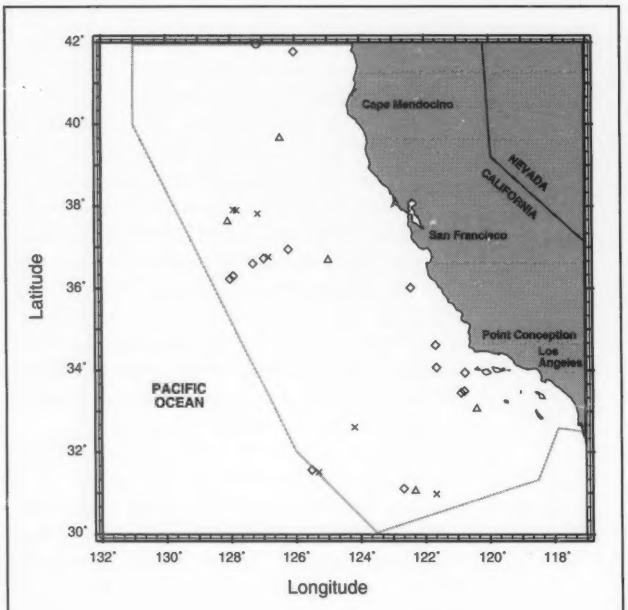

Figure 6

Locations of on-effort sightings of beaked whales of the genus *Mesoplodon* (△), Cuvier's beaked whales (◇), Baird's beaked whales (○), and unidentified beaked whales (×). Scientific names are given in Table 1.

model by minimizing AIC would have been too time consuming to include in the bootstrap procedure; hence, precision estimates are contingent on the chosen models being approximately correct. Variability in estimating mean group size was included implicitly in the Monte Carlo sampling, but it was assumed that the group size estimate for any given group was accurate. Pooling of data to estimate $f(0)$ and $g(0)$ introduces a bias (to the extent that individuals differ within a pooled group) which is not accounted for in precision estimates. All of these factors would tend to result in precision being overestimated. Overall, coefficients of variation are likely to be too small and true confidence intervals are probably wider than those reported.

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Appendix

To estimate the total fraction of trackline groups missed owing to perception bias requires that the survey be designed with two teams of completely independent observers. To be independent, both teams would have to search simultaneously, not notifying or cueing each other until a group of animals had passed abeam of the vessel and were clearly missed by the other team. This approach was deemed infeasible because of the need to approach groups to estimate group size and species composition. If the vessel was not turned until after all groups had passed abeam, a very large percentage of those groups would not be relocated. The probability of relocation would depend on group size and species composition. These factors would add considerably to the difficulty in interpreting such survey data.

Instead, the survey was designed to use a single, conditionally independent observer who was aware of sightings made by the primary team, but who did not reveal the presence of a group until that group was clearly missed by the primary team. Data from the conditionally independent observer are used to make an estimate of the probability that the primary survey team detected a trackline group.

The expected number of groups, n, seen very close to the transect line, say within distance δ, can be estimated as

$$n_\delta = \frac{n_\omega \int_0^\delta g(x)h(x)dx}{\int_0^\omega g(x)h(x)dx}, \quad (3)$$

where n_ω is the total number of groups seen within the truncation distance ω, $g(x)$ is the probability of seeing a group that is at perpendicular distance x, and $h(x)$ is the probability that a group will be at perpendicular distance x (usually assumed to be 1.0 for primary observers at all x). As δ approaches zero distance, the above equation can be reexpressed as

$$n_\delta = \frac{n_\omega g(0)h(0)\delta}{\int_0^\omega g(x)h(x)dx}, \quad (4)$$

which, from the line-transect definition of $f(0)$ (Burnham et al., 1980), can be simplified to

$$n_\delta = n_\omega f(0)\delta. \quad (5)$$

The probability of a trackline group being seen by the primary observers can be expressed as

$$g_1(0) = \frac{n_{1\delta}}{n_{1\delta} + n_{2\delta}/g_2(0)}, \quad (6)$$

where the subscript 1 refers to sightings made by the primary observers and subscript 2 refers to sightings missed by the primary observers but seen by the independent observer. Combining Equations 5 and 6 and simplifying results in

$$g_1(0) = \frac{n_{1\omega} f_1(0)}{n_{1\omega} f_1(0) + n_{2\omega} f_2(0)/g_2(0)}. \quad (7)$$

Because there were three primary observers and only one independent observer, $g_1(0)$ should be greater than or equal to $g_2(0)$. Thus

$$g_1(0) \le 1 - \frac{n_{2\omega} f_2(0)}{n_{1\omega} f_1(0)}. \quad (8)$$

This equation was applied (substituting = for $\le$) to the subset of data collected while an independent observer was on duty to estimate the probability that a group on the trackline would have been seen by the primary observer team. This quantity will be biased and overestimated to the extent that $g_1(0)$ is greater than $g_2(0)$.

The coefficient of variation for $g_1(0)$ can be approximated as

$$CV\left(g_1(0)\right) = \frac{m\,CV(m)}{1-m}, \quad (9)$$

given

$$m = \frac{n_{2\omega} f_2(0)}{n_{1\omega} f_1(0)}, \quad (10)$$

and

$$CV(m) = \sqrt{CV^2(n_{1\omega}) + CV^2(n_{2\omega}) + CV^2\left(f_1(0)\right) + CV^2\left(f_2(0)\right)}. \quad (11)$$

The abundance of cetaceans in California waters. Part II: Aerial surveys in winter and spring of 1991 and 1992

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Abstract.—Two aerial line-transect censuses of cetaceans were conducted along the California coast during March–April 1991 and February–April 1992. The two surveys were designed to provide a combined estimate of cetacean abundance for winter and spring (cold-water) conditions; they complemented a summer and fall ship survey in 1991. The study area (264,270 km^2) extended about 278 km (150 nmi) off the coast of southern California, and 185 km (100 nmi) off the coast of central and northern California. A primary team of two observers searched for cetacean species through bubble windows that allowed an unobstructed view to the sides and directly beneath the aircraft. A third, conditionally independent observer searched through a belly window and reported animals that were missed by the primary team. Approximately 7,069 km and 5,973 km were searched in 1991 and 1992, respectively, resulting in 253 sightings of at least 18 cetacean species (some animals could only be identified to higher taxa). Estimates of abundance and coefficients of variation (in parentheses) for the most common small cetaceans are the following: 306,000 (0.34) common dolphins, *Delphinus* spp.; 122,000 (0.47) Pacific white-sided dolphins, *Lagenorhynchus obliquidens;* 32,400 (0.46) Risso's dolphins, *Grampus griseus*; and 21,300 (0.43) northern right whale dolphins, *Lissodelphis borealis*. Abundance estimates (and CV's) for the most common whales are the following: 892 (0.99) sperm whales, *Physeter macrocephalus*; 392 (0.41) beaked whales, genera *Mesoplodon* and *Ziphius*; 319 (0.41) humpback whales, *Megaptera novaeangliae*; and 73 (0.62) minke whales, *Balaenoptera acutorostrata*.

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California coastal waters are a productive and highly variable oceanographic region with a diverse marine fauna. Coastal fisheries, primarily gillnet fisheries, cause the incidental death of a variety of marine mammal species (Barlow et al., in press). However, the impact of this mortality can only be evaluated if estimates of population size are available for the affected species. In the late 1970's and early 1980's, abundance estimates were obtained based on aerial surveys,[1,2] but estimates of precision were not obtained for most species. Because of the age and uncertainty of these estimates, the National Marine Fisheries Service conducted aerial and shipboard surveys during 1991 and 1992. Based on evidence of seasonality in the abundance and distribution of some cetaceans (Leatherwood and Walker, 1979; Dohl et al., 1986), separate abundance estimates were obtained for winter and summer conditions. Two aerial surveys (March–April 1991 and February–April 1992) were completed during cold-water conditions, and one ship survey (July–November 1991) was conducted during warm-water conditions (Barlow, this issue). The survey periods were chosen based on climatic atlases of the California coast which show that, on average, March and April have the coldest, and September and October the warmest sea-surface temperatures (U.S. Navy, 1977). Standard line-transect methods (Burnham et al., 1980; Buckland et al., 1993a) were used from both platforms. Preliminary abundance estimates were calculated after completion of the first aerial survey in 1991 (Forney and Barlow, 1993), but confidence limits were large. In this paper, we present combined abundance estimates for the 1991 and 1992 aerial surveys.

Survey methods

The methods used during the 1991–92 aerial surveys are described in detail by Forney and Barlow (1993) and Carretta and Forney (1993), and only a summary is presented below. The study area (264,270 km^2)

[1] Dohl, T. P., K. S. Norris, R. C. Guess, J. D. Bryant, and M. W. Honig. 1978. Cetacea of the Southern California Bight. Part II of Summary of marine mammal and seabird surveys of the Southern California Bight area, 1975–1978. Final Report to the Bureau of Land Management, 414 p. [NTIS Rep. PB81248189.]

[2] Dohl, T. P., R. C. Guess, M. L. Duman, and R. C. Helm. 1983. Cetaceans of central and northern California, 1980–1983: status, abundance and distribution. OCS Study MMS 84–0045. Minerals Management Service contract No. 14-12-0001-29090, 284 p.

encompasses California waters out to a distance of 185–278 km (100–150 nmi) from the coast and roughly a depth of 3,000–4,000 m (Fig. 1). It was defined on the basis of the distribution of fisheries that are known to take marine mammals and does not reflect a distributional boundary for any marine mammal population. Surveys were conducted along transect lines forming two nearly uniform, overlapping grids (Fig. 1). The resulting overall grid lines were spaced 41–46 km (22–25 nmi) apart. The location of the transect grid was chosen without reference to specific areas or topographical features. To avoid potential differences in regional coverage, an attempt was made in each year to complete all transects of the first grid, providing coarse coverage of the entire study area, before beginning the second grid. However, in both years, poor weather conditions prevented the completion of both survey grids. In 1991, 85% (5,326 km) of transect grid 1 and 27% (1,739 km) of grid 2 were completed, and in 1992, 81% (5,065 km) of transect grid 1 and 14% (890 km) of grid 2 were completed. The relative proportions of survey effort in different sea state and cloud cover conditions were similar for the two years (Table 1).

The survey platform was a twin-engine turbo-prop DeHavilland Twin Otter, flown approximately at an altitude of 213 m (700 ft) and an airspeed of 165–185 km/h (90–100 knots). All cetacean and sea turtle sightings were recorded, but because of the high densities of pinnipeds near rookeries, these species were recorded only when seen farther than 10 km from land. Two "primary" observers searched through bubble windows on the left and right sides of the aircraft. These windows allowed observers to view to the side and directly beneath the aircraft with at least 10° of overlap between sides. To achieve higher sighting efficiency near the transect line, observers searched for cetaceans only out to a declination angle of 12° (1,004 m perpendicular distance). An additional "secondary" observer monitored the trackline area out to 55° declination angles (on both sides) through a round 45-cm (18-in) viewing hole in the belly of the aircraft and reported sightings missed by the primary team. A fourth person recorded all sighting, effort, and environmental data. To minimize observer fatigue, all observers rotated between these four active positions and one resting position roughly every 30 minutes. All observers had previous experience in identifying cetacean species from aerial or shipboard platforms, or both.

All survey data were recorded on a laptop computer connected to a LORAN or GPS (Global Positioning System) navigational receiver, providing a continuous record of position (updated every few seconds), altitude, air speed, and survey conditions. Environmental conditions, such as Beaufort sea state, percent cloud cover, and glare, were updated whenever changes occurred. Conversation in the aircraft was recorded on a central cassette recorder as a backup to the computer record. Observers also recorded individual sighting information into personal notebooks. Surveys were conducted only in Beaufort sea states 0–4.

Following the methods described in Forney and Barlow (1993) and Carretta and Forney (1993), the aircraft circled for each sighting to obtain species identifications and school size estimates

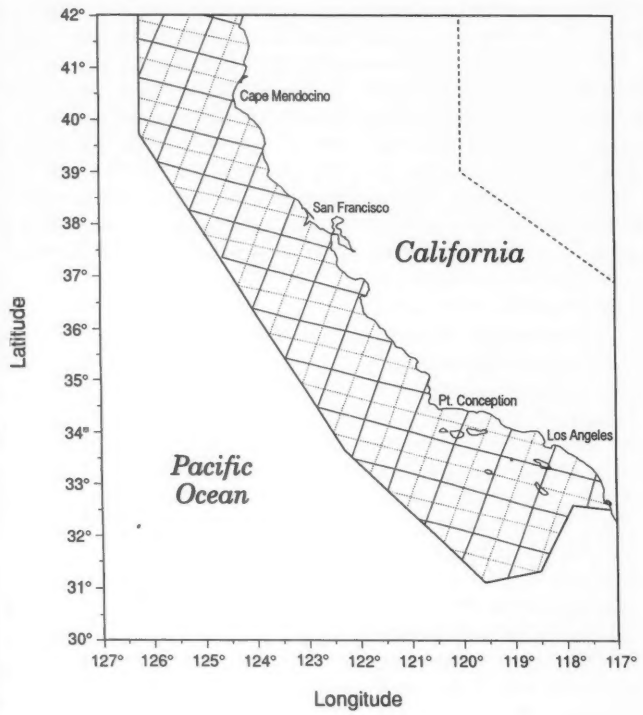

Figure 1

Study area with two overlapping transects grids. The solid line represents grid 1, the dotted line grid 2.

Table 1

Survey effort (in km) stratified by sea state and percent cloud cover.

	Beaufort sea state				
% Cloud cover	0 and 1	2	3	4	Total
1991					
0–24	212	913	1,932	1,346	4,403
25–49	26	66	96	85	273
50–74	45	58	331	241	676
75–100	76	129	980	532	1716
Total	359	1,166	3,338	2,205	7,069
1992					
0–24	406	933	1,349	1,220	3,908
25–49	0	8	141	113	262
50–74	2	43	192	47	284
75–100	78	251	758	433	1,519
Total	486	1,235	2,440	1,813	5,973
Both years combined					
0–24	618	1,846	3,280	2,566	8,311
25–49	26	74	238	199	536
50–74	47	101	523	288	960
75–100	154	380	1,737	965	3,235
Total	845	2,401	5,778	4,018	13,042

(each observer made a confidential record of best, high, and low estimate into a personal field notebook). Any additional schools sighted while the aircraft was diverted from the transect were recorded as 'off-effort' sightings. Only sightings made during active searches on predetermined transect lines ('on-effort') were included for abundance estimation. The secondary observer only reported sightings missed by the primary observer team; these secondary sightings were used to estimate the fraction of animals missed on the transect line.

Analytical methods

Stratification

Because we were not able to complete both grids in all regions of the coast, the study area was divided into four a posteriori geographic areas to approximate uniform coverage within each stratum (Fig. 2). Environmental conditions such as sea state and percent cloud cover were recorded throughout the survey, as they have been shown to influence cetacean sighting rates (Holt and Cologne, 1987; Forney et al., 1991). However, because of the small number of sightings made during each combination of environmental conditions, it was not possible to evaluate their effect quantitatively.

Because of the difficulty in identifying beaked whales to species level during aerial surveys, only a combined abundance estimate was obtained for this group. In the preliminary analyses of the 1991 aerial survey data, Forney and Barlow (1993) assigned other unidentified species based on a 'nearest identified neighbor' approach. In the analyses presented here, unidentified cetacean sightings were treated separately as either 'unidentified dolphin or porpoise,' 'unidentified small whale,' or 'unidentified large whale,' because they represented only a small fraction of the total animals seen.

The small number of sightings for each species made it necessary to pool distributions of perpendicular sighting distances for line-transect calculations. Forney and Barlow (1993) created preliminary species groups based on considerations of school size, body size and behavior, and pooled distributions for groups that were not statistically different from one another. The same procedure was used for this analysis, resulting in the same three species/group-size categories: 1) small cetacean groups with 1–10 animals; 2) small cetacean groups with more than 10 animals; and 3) medium and large cetaceans (Table 2).

Table 2

Estimates of $f(0)$ and $g(0)$, and number of sightings (n) for the three species/group-size categories used in the analysis.

Small cetaceans	Group size	n	$f(0)$	$g(0)$
	1–10	99	4.70	0.67
	> 10	53	2.85	0.85
Species				
Harbor porpoise, *Phocoena phocoena*				
Dall's porpoise, *Phocoenoides dalli*				
Pacific white-sided dolphin, *Lagenorhynchus obliquidens*				
Risso's dolphin, *Grampus griseus*				
Bottlenose dolphin, *Tursiops truncatus*				
Common dolphins *Delphinus delphis* and *D. capensis*				
Northern right whale dolphin, *Lissodelphis borealis*				

Medium and large cetaceans	Group size	n	$f(0)$	$g(0)$
	1–22	57	2.49	0.95
Species				
Killer whale, *Orcinus orca*				
Small beaked whales, *Ziphius cavirostris* and *Mesoplodon* spp.				
Sperm whale, *Physeter macrocephalus*				
Right whale, *Eubalaena glacialis*				
Gray whale, *Eschrichtius robustus*				
Minke whale, *Balaenoptera acutorostrata*				
Blue whale, *B. musculus*				
Fin whale, *B. physalus*				
Humpback whale, *Megaptera novaeangliae*				

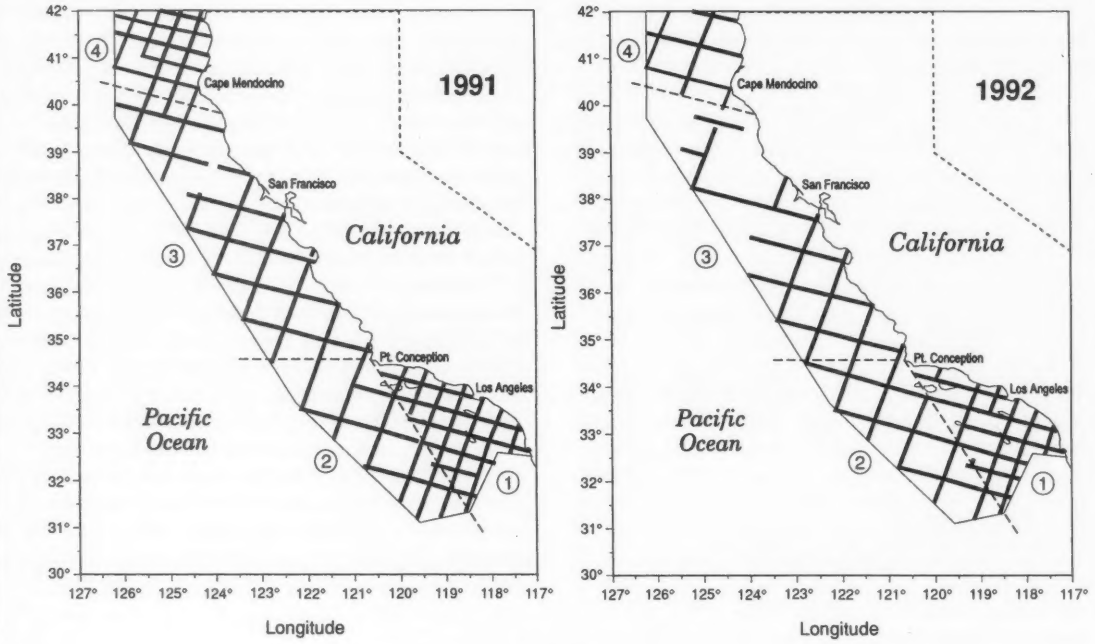

Figure 2

Completed transects (solid lines) for 1991 and 1992, and a posteriori geographic strata (separated by broken lines) used in the analysis. Area numbers are shown in circles.

Abundance estimation

Line transect methods (Burnham et al., 1980; Buckland et al., 1993a) were applied to estimate abundances separately for each species in each stratum:

$$N_k = \sum_{j=1}^{3} \sum_{i=1}^{4} \frac{A_i\, n_{i,j,k}\, S_{i,j,k}\, f_j(0)}{2 L_i\, g_j(0)}, \qquad (1)$$

where

N_k = estimated total number of animals of species k in the study area;
$n_{i,j,k}$ = number of sightings of species k in area i and species/group-size category j;
$s_{i,j,k}$ = average group size of species k in area i and species/group-size category j, calculated as the total number of animals in all groups divided by the number of groups sighted;
$f_j(0)$ = the probability density function evaluated at zero perpendicular distance for species/group-size category j;
$g_j(0)$ = the probability of detecting a group of animals on the transect line for species/group-size category j;
L_i = the length of transect surveyed in area i (in km); and
A_i = the size of area i (in km^2).

Values for $f(0)$ were obtained for each species/group-size category by fitting the distribution of all perpendicular sighting distances (primary and secondary; measured in km) to the Hazard rate model with the statistical software program HAZARD (Buckland, 1985). A value for $g(0)$ was estimated following the methods described in Forney and Barlow (1993), but because of small sample sizes, it was not possible to estimate the variance in $g(0)$. This should result in a downward bias in the variance of the abundance estimates, but bias in the abundance estimates themselves will be reduced. The lengths of transect lines flown, L_i (and total sizes, A_i), for the four areas are 3,715 km (46,300 km^2) for area 1; 2,831 km (63,772 km^2) for area 2; 4,461 km (120,108 km^2) for area 3; and 2,035 km (34,090 km^2) for area 4.

Variance estimation

Variance in estimated abundance was calculated with bootstrap techniques applied to the complete data

set. The data were subdivided by area into effort segments of equal length, and the segments were then drawn randomly with replacement until the total number of kilometers actually surveyed in each area was reached. This process was replicated 1,000 times. Forney and Barlow (1993) demonstrated that the choice of segment lengths between 5 km and 20 km did not influence the resulting estimates of precision. In this analysis we also performed bootstrap simulations for 50 km and 100 km segments and again found that segment length did not affect estimates of variance. For the bootstrap analysis, we chose a segment length of 50 km, which roughly reflects the degree of sampling variability for these surveys (i.e. the dimension of actual gaps in the sampling grid in Figure 2).

Each of the 1,000 bootstrap replicates was treated and analyzed as a separate survey: sightings were first stratified into the three species/group-size categories given above. Individual values for n and s were calculated, and $f(0)$ was estimated with the program HAZARD. The estimated value of $g(0)$ was treated as a correction factor known without error. The variance, coefficient of variation, and 95% confidence intervals were obtained from the distribution of the 1,000 bootstrap abundance estimates with standard formulae. Because the bootstrap method (Buckland, 1984) of obtaining confidence intervals can result in the lower 95% confidence intervals being smaller than the actual number of animals seen (or even zero) we also calculated log-normal confidence intervals based on the bootstrap coefficient of variation.

Results

Detailed results of the survey, including sighting information and plots of sighting locations for all species sighted are presented elsewhere (Carretta and Forney, 1993). Results relevant to the analyses presented in this paper are given below. A total of 253 cetacean sightings were made (Fig. 3): 213 on effort (while actively searching), and an additional 40 off effort (24 while in transit, 8 beyond 12° declination angle, 7 while circling over another group of animals, and 1 by an off-effort observer). Twenty eight on-effort sightings could not be positively identified to the

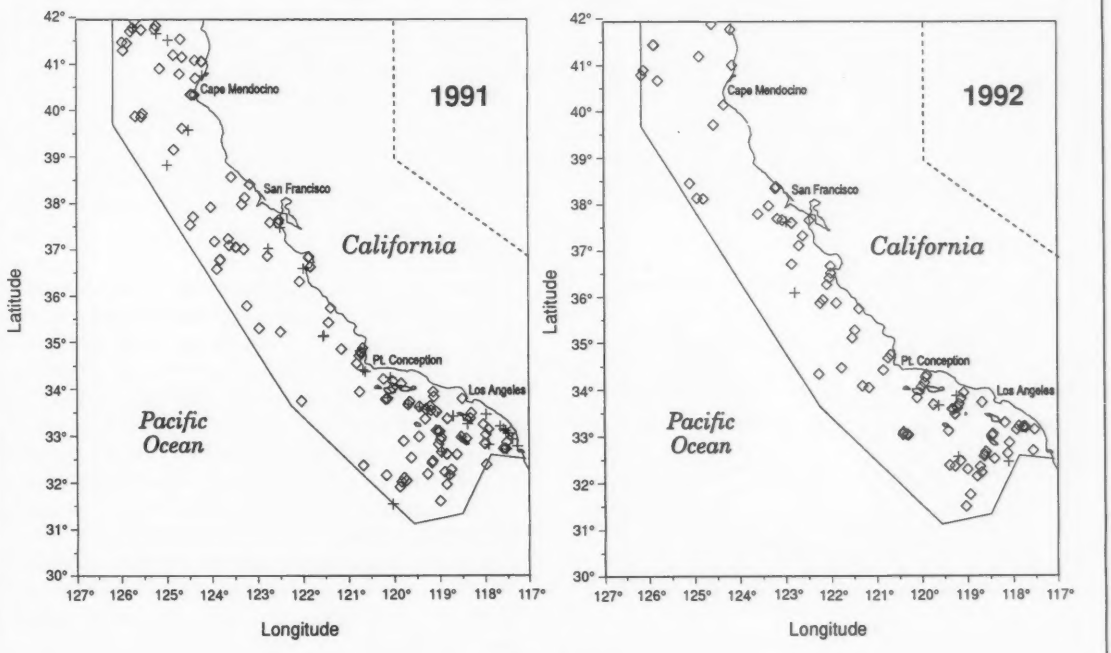

Figure 3

Locations of all 253 cetacean sightings made during the 1991 and 1992 surveys. The 213 on-effort sightings (used in the abundance estimation) are shown by diamonds, and the 40 off-effort sightings (e.g. made while circling or in transit) are shown with plus signs.

species level. Four of these sightings were identified as ziphiid whales, for which a combined abundance estimate was calculated. The remaining 24 sightings were treated separately in the analyses.

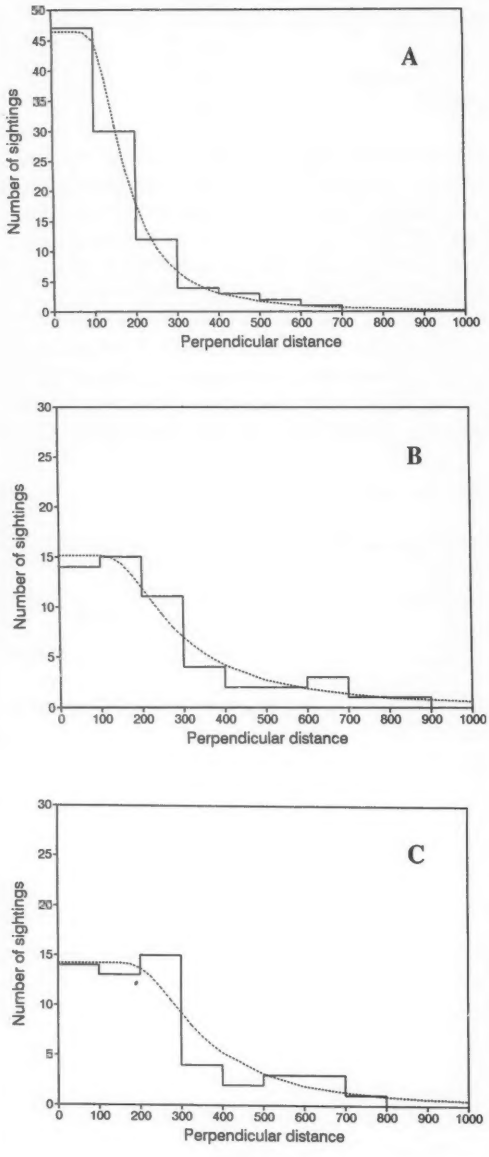

Figure 4

Distribution of perpendicular sighting distances (100-m intervals; solid line) and Hazard model fit (dotted line) for (**A**) small cetaceans in groups ≤10, (**B**) small cetaceans in groups >10, and (**C**) medium and large cetaceans.

The Hazard model provided adequate fits to the perpendicular distance distributions for the three species/group-size categories (Fig. 4). Estimates of $f(0)$ and $g(0)$ are given for each group in Table 1. Although the full transect grid was not completed in either year because of poor weather, the resulting estimates of abundance (Table 3) are the most precise that have been produced to date for this area and season. CV's range from 0.24 to 0.49 for small cetaceans and from 0.35 to 1.11 for large cetaceans.

Discussion

Comparisons with previous abundance estimates

Our abundance estimates (Table 3) can be compared directly with estimates based on 1975–83 aerial surveys,[1,2] which are likely to have similar biases. The estimate of 8,460 Dall's porpoise, *Phocoenoides dalli*, is similar to previous aerial survey estimates of 3,000–4,000 in winter and spring.[1,2] The current estimate of 122,000 Pacific white-sided dolphins,[3] *Lagenorhynchus obliquidens*, is greater than the combined estimates of 26,000 (spring) to 33,500 (winter) for central and northern California[2] and 5,300 (Jan–Jun) for southern California.[1] Our estimate of 21,300 northern right whale dolphins is less than the combined estimates of 29,000 (spring) to 61,500 (winter) for central and northern California[2] and 5,900 (Jan–Jun) for southern California.[1] The prior studies do not give estimates of statistical precision for any of the above species, but given the CV's of our estimates, the above differences are not likely to be statistically significant.

In contrast to the species above, common dolphins, *Delphinus* spp., appear to be much more abundant at present than during the period 1975–83. The current winter estimate (306,000; CV=0.34) is more than an order of magnitude larger than the previous value of 15,488 (CV=0.36; Dohl et al., 1986), and the 99% log-normal confidence limits for these two estimates do not overlap. Preliminary comparisons (Barlow, unpubl. data) of 1979 and 1980 ship surveys with the 1991 ship survey (Barlow, this issue) also show a significant increase in common dolphin abundance. Based on these two separate lines of evidence for winter and summer conditions, the abundance of common dolphins in California appears to have in-

[3] Although estimates for Pacific white-sided dolphins based on the combined 1991 and 1992 survey data are over twice the preliminary estimate of 46,000 from only the 1991 data (Forney and Barlow, 1993), the new estimate lies well within the 95% confidence limit of the previous value.

Table 3

Number of groups seen, mean group size, density of individuals, and abundance estimates for cetaceans in the entire California study area, and subdivided by geographic stratum (See Fig. 2). Coefficients of variation (CV) and 95% confidence intervals (CI) for the overall abundance estimates are also given. Unid.=unidentified.

Species and area	Number of groups	Mean group size	Animal density km^{-2}	Population size N	CV	Bootstrap CI		Log-normal CI	
						Lower 95%	Upper 95%	Lower 95%	Upper 95%
Harbor porpoise[1]	18	1.2	0.0060	1,599	0.345	664	2,915	829	3,085
Area 1	0	0.0	0.0000	0					
Area 2	0	0.0	0.0000	0					
Area 3	10	1.0	0.0079	949					
Area 4	8	1.4	0.0191	650					
Dall's porpoise	38	3.1	0.0320	8,460	0.240	5,203	13,361	5,320	13,453
Area 1	9	4.0	0.0342	1,582					
Area 2	2	4.5	0.0112	716					
Area 3	19	2.6	0.0395	4,744					
Area 4	8	3.0	0.0416	1,418					
Pacific white-sided dolphin	21	151.6	0.4605	121,693	0.466	35,404	261,524	51,041	290,144
Area 1	5	24.6	0.0573	2,654					
Area 2	7	69.4	0.2945	18,779					
Area 3	7	237.1	0.6218	74,678					
Area 4	2	457.0	0.7505	25,583					
Risso's dolphin	19	47.6	0.1225	32,376	0.456	10,255	65,984	13,812	75,891
Area 1	14	28.5	0.2029	9,396					
Area 2	1	8.0	0.0100	636					
Area 3	4	124.3	0.1860	22,343					
Area 4	0	0.0	0.0000	0					
Bottlenose dolphin	8	17.9	0.0123	3,260	0.487	618	6,783	1,320	8,052
Area 1	7	20.3	0.0684	3,165					
Area 2	0	0.0	0.0000	0					
Area 3	1	1.0	0.0008	95					
Area 4	0	0.0	0.0000	0					
Common dolphins	27	514.9	1.1568	305,694	0.340	124,730	539,319	159,864	584,552
Area 1	22	592.7	5.8769	272,101					
Area 2	4	176.0	0.4161	26,535					
Area 3	1	157.0	0.0588	7,058					
Area 4	0	0.0	0.0000	0					
Northern right whale dolphin	31	18.9	0.0807	21,332	0.428	9,151	42,629	9,548	47,658
Area 1	18	12.3	0.1378	6,381					
Area 2	4	56.5	0.1395	8,895					
Area 3	6	11.8	0.0341	4,091					
Area 4	3	22.7	0.0577	1,966					
Killer whale	2	1.0	0.0002	65	0.689	0	133	19	220
Area 1	0	0.0	0.0000	0					
Area 2	1	1.0	0.0005	30					
Area 3	1	1.0	0.0003	35					
Area 4	0	0.0	0.0000	0					
Beaked whales[2]	8	1.9	0.0015	392	0.408	151	774	182	845
Area 1	0	0.0	0.0000	0					
Area 2	3	1.0	0.0014	89					
Area 3	2	1.5	0.0009	106					
Area 4	3	3.0	0.0058	197					
Sperm whale	3	10.0	0.0034	892	0.990	0	2,798	176	4,506
Area 1	0	0.0	0.0000	0					
Area 2	2	14.5	0.0134	857					

Table 3 (Continued)

Species and area	Number of groups	Mean group size	Animal density km^{-2}	Population size N	CV	Bootstrap CI		Log-normal CI	
						Lower 95%	Upper 95%	Lower 95%	Upper 95%
Area 3	1	1.0	0.0003	35					
Area 4	0	0.0	0.0000	0					
Northern right whale	1	1.0	0.0001	16	1.110	0	59	3	95
Area 1	1	1.0	0.0004	16					
Area 2	0	0.0	0.0000	0					
Area 3	0	0.0	0.0000	0					
Area 4	0	0.0	0.0000	0					
Gray whale[3]	25	4.2	0.0108	2,844	0.347	1,187	5,270	1,469	5,507
Area 1	12	3.4	0.0145	669					
Area 2	0	0.0	0.0000	0					
Area 3	11	5.3	0.0170	2,043					
Area 4	2	3.0	0.0039	132					
Minke whale	3	1.0	0.0003	73	0.616	0	181	24	223
Area 1	1	1.0	0.0004	16					
Area 2	0	0.0	0.0000	0					
Area 3	1	1.0	0.0003	35					
Area 4	1	1.0	0.0006	22					
Blue whale	1	1.0	0.0001	30	0.990	0	100	6	149
Area 1	0	0.0	0.0000	0					
Area 2	1	1.0	0.0005	30					
Area 3	0	0.0	0.0000	0					
Area 4	0	0.0	0.0000	0					
Fin whale	2	1.5	0.0002	49	1.012	0	57	9	254
Area 1	2	1.5	0.0011	49					
Area 2	0	0.0	0.0000	0					
Area 3	0	0.0	0.0000	0					
Area 4	0	0.0	0.0000	0					
Humpback whale	8	1.6	0.0012	319	0.407	114	622	148	688
Area 1	1	1.0	0.0004	16					
Area 2	0	0.0	0.0000	0					
Area 3	2	1.5	0.0009	106					
Area 4	5	1.8	0.0058	197					
Unid. large whale	5	1.2	0.0006	160	0.457	40	348	68	376
Area 1	1	2.0	0.0007	33					
Area 2	0	0.0	0.0000	0					
Area 3	3	1.0	0.0009	106					
Area 4	1	1.0	0.0006	22					
Unid. small whale	3	1.0	0.0003	68	0.676	0	188	20	226
Area 1	2	1.0	0.0007	33					
Area 2	0	0.0	0.0000	0					
Area 3	1	1.0	0.0003	35					
Area 4	0	0.0	0.0000	0					
Unid. dolphin or porpoise	15	4.4	0.0180	4,766	0.331	2,050	8,368	2,533	8,966
Area 1	2	1.5	0.0028	132					
Area 2	5	4.2	0.0223	1,419					
Area 3	7	5.7	0.0258	3,096					
Area 4	1	2.0	0.0035	118					

[1] More appropriate estimates for harbor porpoise are recently available in Barlow and Forney (1994). (See Discussion section.)

[2] This category includes beaked whales of the genus *Mesoplodon* and Cuvier's beaked whale, *Ziphius cavirostris*. No Baird's beaked whales, *Berardius bairdii*, were seen during the surveys.

[3] A more accurate estimate of the entire population of California gray whales is presented in Buckland et al., 1993. (See Discussion section.)

creased dramatically since the early 1980's. The causes of this increase are not known, but it is possible that long-term oceanographic changes (Roemmich, 1992; Roemmich and McGowan, 1994) have resulted in a shift in the distribution of common dolphins into this area. This hypothesis is consistent with the observed decline in population size of the northern common dolphin south of our study area (Anganuzzi and Buckland, 1994).

Similarly, an apparent decrease in abundance was seen in short-finned pilot whales, *Globicephala macrorhynchus*. This species was commonly seen in the Southern California Bight on surveys during the late 1970's and early 1980's,[1,2] but only one off-effort sighting of four animals was made during our surveys.

Our estimate of 304 humpback whales is roughly half the recent estimate obtained from photo-identification studies.[4] This is quite surprising because humpback whales, *Megaptera novaeangliae*, in the California feeding population are expected to be in waters off Mexico during the winter and spring season. However, it is possible that some animals had already moved north into California at the time of the sightings. Alternatively, the sighted animals may have been part of the southeastern Alaska feeding population that migrates southward to breed in Mexican waters in spring (Baker et al., 1986).

Previously published estimates for harbor porpoise, *Phocoena phocoena* (Barlow, 1988; Barlow et al., 1988; Barlow and Forney, 1994) and gray whales, *Eschrichtius robustus* (Reilly, 1984; Buckland et al., 1993b), are substantially higher than the estimates presented here. This is probably because the defined study area is not appropriate for the range of these animals. Gray whales have a much larger range and migrate through California waters (southward and then northward) from roughly November to May. Our estimate represents that portion of the population which was migrating through California in March and early April. Harbor porpoise are limited to a narrow coastal band, and our transect lines only overlapped with this region at specific points. More appropriate abundance estimates for harbor porpoise are published in Barlow (1988) and in Barlow and Forney (1994).

Comparisons with 1991 ship surveys

Although a statistical comparison between these winter and spring aerial survey estimates and the 1991 summer and fall ship survey estimates (Barlow, this issue) is precluded at this time because of differences in the sizes of the two study areas, a few patterns are noteworthy. Despite the differences in seasonal timing and areal coverage, estimates of abundance are very similar for several species. Similar estimates of abundance were obtained for total common dolphins (306,000 vs. 246,000), northern right whale dolphins, *Lissodelphis borealis* (21,300 vs. 9,340), bottlenose dolphins, *Tursiops truncatus* (3,260 vs. 1,500), and sperm whales, *Physeter macrocephalus* (892 vs. 756) (aerial vs. ship estimates, respectively). More disparate estimates were obtained for Pacific white-sided dolphins (122,000 vs. 12,300), Risso's dolphins, *Grampus griseus* (32,400 vs. 8,500), harbor porpoise (1,600 vs. 52,700), Dall's porpoise (8,460 vs. 78,400), and total beaked whales, *Ziphius cavirostris* and *Mesoplodon* spp. (392 vs. 3,230).

It may be important to note that all cases in which the ship estimates are substantially larger than the aerial estimates are for species which spend a large fraction of their time diving (harbor porpoise, Dall's porpoise, and beaked whales). Such species could be more easily missed by aerial observers owing to availability bias. In the case of Pacific white-sided dolphins and Risso's dolphins, the winter and spring aerial estimates may be larger because of a seasonal movement of animals out of Oregon and Washington in winter.[5] Additional analyses, which account for differences in geographic extent of the aerial vs. ship surveys, are planned in the future.

Bias

There are several sources of potential bias in this study. First, abundance estimates may be biased low because animals are missed by aerial observers (perception bias; Marsh and Sinclair, 1989). This is most likely to be a problem with poor observation conditions (high sea state or overcast conditions, or both). We have attempted to estimate the magnitude of perception bias in this study through the use of a conditionally independent observer and have corrected abundance estimates to reduce this effect. A second source of downward bias, availability bias (Marsh and Sinclair, 1989), is introduced because animals that are submerged when the aircraft passes overhead are not available to be seen. This effect is

[4] Calambokidis, J., G. H. Steiger, and J. R. Evenson. 1993. Photographic identification and abundance estimates of humpback and blue whales off California in 1991–92. Final Contract Report 50ABNF100137 to Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038, 67 p.

[5] Green, G. A., J. J. Brueggeman, R. A. Grotefendt, C. E. Bowlby, M. L. Bonnell, and K. C. Balcomb III. 1992. Cetacean distribution and abundance off Oregon and Washington, 1989–1990. Ch. 1 *in* J. J. Brueggeman (ed.), Oregon and Washington marine mammal and seabird surveys. Minerals Management Service Contract Report 14-12-0001-30426 prepared for the Pacific OCS (Outer Continental Shelf) Region.

expected to be smallest for species which tend to occur in large groups, such as common dolphins, and largest for species which spend relatively little time at the surface, such as porpoise, beaked whales, and sperm whales.

Dive studies (Barlow et al., 1988) may provide information on the magnitude of availability bias, but each species requires a separate assessment of the average proportion of time it spends at the surface (and hence is 'available'), and adequate estimates are not currently available for most species in California waters. Rough estimates can be made for Dall's porpoise and humpback whales based on prior studies. Dall's porpoise have similar sighting characteristics to those of harbor porpoise (both have a small body size and generally are found in small groups); thus, assuming that dive patterns are similar and applying the correction factor of 3.1 (CV=0.17) for harbor porpoise,[6] one would obtain a corrected estimate of approximately 26,200 Dall's porpoise. Based on a very small sample, a correction factor of 2.7 has been estimated for humpback whales.[7] This would yield a corrected abundance estimate of 861 humpback whales. Clearly, given the magnitude of these correction factors, availability bias can be substantial.

Potential upward bias in line-transect analysis can result if factors other than distance to the trackline affect the probability of seeing a school. School size has been shown to affect the probability of detection (Drummer, 1985; Holt and Sexton, 1989), and this can lead to an upward bias in the abundance estimate (Quinn, 1985; Drummer and McDonald, 1987; Buckland et al., 1993a). To counteract this effect, we have stratified small cetacean sightings by group size and estimated abundances separately for small and large groups of the same species. This is an artificial separation, but it reduces potential biases that are due to large variation in group size within a single species, such as common dolphins or Pacific white-sided dolphins. Within each stratum, correlations of perpendicular sighting distance with group size are weak and not significant at α=0.05 (r=0.195 for small cetaceans in groups of 1–10 animals; r=0.169 for small cetaceans in groups of greater than 10 animals; and r=0.183 for whales in groups of all sizes).

[6] Calambokidis, J., J. R. Evenson, J. C. Cubbage, P. J. Gearin, and S. D. Osmek. 1993. Development of a correction factor for aerial surveys of harbor porpoise. Draft Final Contract Report to the National Marine Mammal Laboratory, NMFS, NOAA, 7600 Sand Point Way NE, BIN C-15700, Seattle, WA 98115. 36 p.

[7] Calambokidis, J., G. H. Steiger, J. C. Cubbage, K. C. Balcomb, and P. Bloedel. 1989. Biology of humpback whales in the Gulf of the Farallones. Final report for Contract CX-8000-6-0003 to Gulf of the Farallones National Marine Sanctuary, NOAA, Fort Mason Center, Bldg. 201, San Francisco, CA 94123, 93 p.

In summary, we have attempted to correct for perception bias by estimating the fraction of animals missed during these surveys and have minimized potential upward bias with a poststratification by school-size range. However, species-specific availability bias cannot currently be estimated, and overall our abundance estimates are likely to be biased downward.

Precision

Estimation of variance for line-transect abundance calculations can be difficult. We have attempted to include most of the sources of sampling error in the bootstrap procedure, which reestimates n, s, and $f(0)$ (in Eq. 1) for each replicate. Our analysis revealed that the choice of segment length used for the bootstrap did not affect the resulting estimates of precision within the range of appropriate segment lengths for this study (5–100 km; longer segments would not be appropriate because surveys extended only 100–150 km offshore). However, potential heterogeneity due to the pooling of different species and group sizes for estimation of $f(0)$ and $g(0)$ was not accounted for in precision estimates. Furthermore, we did not include the variance in $g(0)$ or in the estimation of group size for each school encountered (however, the variance in the estimated mean group size for the survey was included in the bootstrap procedure). Thus, the coefficients of variation for the abundance estimates (Table 3) are likely to be underestimated and the confidence intervals are likely to be too narrow.

Considerations for future aerial surveys

Two species of common dolphins, short-beaked and long-beaked, are recognized in California waters (Rosel, 1992; Dizon et al., 1994; Heyning and Perrin, 1994). Although clear differences in color pattern, size, and beak length exist between these two forms, it is not currently possible to differentiate them during aerial surveys; therefore the abundance estimate here is a combined estimate. Unless reliable means of identifying the two species from the air are developed, aerial surveys will not be adequate for future assessments requiring separate estimates of short-beaked and long-beaked common dolphins.

Similarly, it was difficult to distinguish between the smaller species of beaked whales during our aerial surveys. The estimates presented for the beaked whales as a group are therefore a combined estimate for *Ziphius cavirostris* and *Mesoplodon* spp. All unidentified beaked whale sightings could be narrowed down to these two genera. The only other beaked whale species known to occur in this region, *Berardius bairdii*, can be readily distinguished based

on its size and was not sighted during this survey. It is likely that the categorization of "small beaked whales" will be necessary on future aerial surveys.

The survey grid used here was not designed for species which are restricted to a narrow coastal region. Harbor porpoise are found primarily in waters inshore of the 50-fathom (92-m) isobath (Barlow, 1988). Two distinct populations of bottlenose dolphins are found in California; the inshore form is found only within about 1 km of shore (Hansen, 1990; NMFS[8]). All of the bottlenose dolphins seen during this aerial survey were at least several miles from the mainland; therefore our estimate is assumed to represent the population of offshore animals. Precise estimates of abundance for harbor porpoise and inshore bottlenose dolphins will require dedicated aerial surveys designed for those species. Work is currently in progress on both of these projects.[8]

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Larval development of King George whiting, *Sillaginodes punctata*, school whiting, *Sillago bassensis*, and yellow fin whiting, *Sillago schomburgkii* (Percoidei: Sillaginidae), from South Australian waters

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Abstract.—The larval development of *Sillaginodes punctata*, *Sillago bassensis*, and *Sillago schomburgkii* is described based on both field-collected and laboratory-reared material. Larvae of the three species can be separated based on a combination of pigment and meristic characters, including extent and appearance of dorsal midline pigment, lateral pigment on the tail, presence or absence of pigment above the notochord tip, myomere number, extent and timing of gut coiling, and size at flexion. The most useful meristic character across the range of specimens was number of myomeres. *Sillaginodes punctata* with 42–45 myomeres are easily distinguished from *Sillago schomburgkii* with 36–38, and from *S. bassensis* with 32–35. The timing of gut coiling and its subsequent effect on anus position differed both among the three species examined here and from that previously reported for sillaginid larvae in general. Timing of gut coiling and extent of anus migration are not useful characters for the identification of temperate Australian sillaginids at the family level but are useful on a specific level. Possible implications of the development of the gut to diet are discussed.

Based on the presence of larvae, all three species spawn in South Australian waters. No larvae of a fourth sillaginid species, *S. flindersi*, were found during the study. South Australia is the western distributional limit for *S. flindersi* and it does not appear to spawn in the area.

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The perciform family Sillaginidae (whiting and sand smelts) consists of three genera, three subgenera, and thirty-one species of small to moderately sized fishes found primarily in shallow coastal waters of the Indo-Pacific (McKay, 1992). Sillaginids are highly valued food fishes in many tropical and temperate waters. The Sillaginidae are related to the Percidae, Sciaenidae, and, to a lesser extent, the Haemulidae (McKay, 1985) although their sister group is yet to be determined (McKay, 1992). The most speciose of the three sillaginid genera (*Sillago*) includes twenty-nine species. The remaining two genera, *Sillaginodes* and *Sillaginopsis*, are monotypic. The taxonomy of the family is approaching stability; only a few species remain undescribed (McKay, 1992).

Two genera and thirteen species of sillaginids are found in Australian waters. Four species inhabit the waters off South Australia: the King George or spotted whiting, *Sillaginodes punctata*; yellow fin whiting, *Sillago schomburgkii*; western school whiting, *Sillago bassensis*; and eastern school whiting, *Sillago flindersii*. The latter two species were, until recently, considered subspecies of *S. bassensis* (McKay, 1992). All four species are widely distributed in southern Australia and form the basis for important commercial fisheries across their range (McKay, 1985; Kailola et al., 1993; May and Maxwell[1]).

The adult and juvenile biology of each of the four species has previously been documented by several authors (Scott, 1954; Gilmour, 1969; Lennanton, 1969; Robertson, 1977; Weng, 1983, 1986; Burchmore et al., 1988; Jones[2]; Jones et al.[3]), but very little is known of their early life history and neither the eggs nor the larvae of any of the four species have previously been described.

In 1986, the South Australian Department of Fisheries began an ichthyoplankton program to inves-

[1] May, J. L., and J. G. H. Maxwell. 1986. Field guide to trawl fish from temperate waters of Australia. CSIRO Division of Fisheries Res., Hobart, Tasmania, 492 p.

[2] Jones, G. K. 1979. Biological investigations on the marine scale fishery in South Australia. South Australian Dep. Agric. and Fisheries Rep., 72 p.

[3] Jones, G. K., D. A. Hall, K. L. Hill, and A. J. Staniford. 1989. The South Australian marine scale fishery: stock assessment, economics, management. South Australian Dep. Fisheries Green Paper, 186 p.

tigate the larval ecology of commercially important fishes of South Australian waters. An important prerequisite of any such program is the ability to make an accurate identification of larvae to species. This paper details the development of *Sillaginodes punctata*, *Sillago schomburgkii*, and *S. bassensis* larvae collected during this study.

Materials and methods

Specimens were obtained from plankton and beach seine samples collected between March 1986 and March 1991 aboard the research vessel MRV *Ngerin* in coastal waters and at various inshore nursery areas off South Australia. Details of sampling locations and procedures are described in Bruce (1989). Briefly, larvae were obtained from stepped oblique tows with 70-cm-diameter bongo nets fitted with 500-micron mesh. Postsettlement (refer to definition below) larvae and juveniles were captured with a fine mesh beach seine (7 m × 1.8 m, 2-mm mesh) as well as by dipnetting and diving. The field-collected series of *Sillaginodes punctata* was supplemented with larvae reared in the laboratory at West Beach, Adelaide.

All field-collected specimens used for description were fixed in a 10% formalin-seawater solution buffered with sodium tetraborate (borax) and were later transferred to a 5% solution buffered with sodium B-glycerophosphate (0.5 g per 1,000 mL). Reared larvae were fixed immediately in the 5% solution. Reared *S. punctata* were used for illustration when possible because of their superior condition. Some pigment differences were apparent between reared and field-collected larvae largely as a result of expansion or contraction of melanophores. Melanophores of field-collected larvae were generally less expanded than reared specimens. Reared larvae were typically greater in length than similarly developed field-collected material owing to increased shrinkage in the latter. Similar shrinkage effects have been previously reported for a variety of species (Theilacker, 1980; Hay, 1981; Bruce, 1988). Unless specified, development at length refers to field-collected material.

Representative series of *S. punctata* and *Sillago bassensis* are deposited with the I.S.R. Munro Fish Collection, CSIRO, Hobart Tasmania. Too few *S. schomburgkii* larvae were collected to allow a complete analysis and all are currently held in a collection maintained by the author at CSIRO Division of Fisheries, Hobart, Tasmania.

Developmental terminology and body measurements follow Leis and Trnski (1989). The term "postsettlement" is used to describe newly settled individuals prior to the acquisition of scales and juvenile colour patterns, after which they are referred to as juveniles. Body length measurements (BL) are measured as notochord length, NL (i.e. from the snout tip to the end of the notochord), in preflexion and flexion larvae, and standard length, SL (i.e. from the snout tip to the posterior margin of the superior hypural elements), in postflexion larvae and juveniles. Body depth is taken at two points. Body depth at pectoral (BDp) is equivalent to "body depth" as defined by Leis and Trnski (1989), that is, as "the vertical distance between body margins (exclusive of fins) through the anterior margin of the pectoral fin base." Body depth at anus (BDa) is defined as the vertical distance between body margins (exclusive of fins and, initially, the gut) through the midpoint of the anal opening. BDa includes the gut only after overlying musculature has developed. *Sillaginodes punctata* eggs were measured with a Zeiss photomicroscope III fitted with an ITC 510 video camera and linked to an Apple Macintosh SE computer via an HEI 582A video coordinate digitizer. Egg dimensions are reported to the nearest micron. Larvae were measured to the nearest 0.1 mm with a dissecting microscope fitted with an ocular micrometer. Postsettlement larvae and juveniles were measured to the nearest 0.1 mm with vernier calipers.

Meristic counts were made on *S. punctata* and *Sillago bassensis* specimens cleared and stained with alcian blue and alizarin red-S following Potthoff (1984). Insufficient specimens of *S. schomburgkii* were available for clearing and staining and therefore all meristics were taken from unstained material.

Descriptions are based primarily on the detailed examination of a representative series of specimens; however, comments on pigment and meristic variability stem from the routine examination of all larvae collected. The number of specimens examined in detail, the size range covered, and the museum reference numbers (for lodged material) are provided under each species account.

Results

Identification

Larvae were identified to family level from larval sillaginid characters reported in the literature. Sillaginid larvae are elongate and have 30–44 myomeres (Johnson, 1984; Miskiewicz, 1987; Leis and Trnski, 1989). The gut typically reaches to greater than 55% body length in preflexion larvae. The anus is reported to migrate anteriorly during development (often during flexion) as a result of coiling of the anterior section of the gut, thus shortening the preanal length

(Leis and Trnski, 1989). Sillaginids have a characteristic series of melanophores along the dorsal and ventral midlines (particularly prominent in small larvae) and generally have pigment located on the angle of the lower jaw.

Three types of sillaginid larvae were found during this study. Specific identity of two of the types (*S. schomburgkii* and *Sillaginodes punctata*) was established by comparing vertebral counts and fin meristics of postflexion larvae to those of adult and juvenile specimens. Smaller larvae were linked by establishing a developmental series based on the extent and appearance of dorsal midline pigment, lateral pigment on the tail, presence or absence of pigment above the notochord tip, myomere number, extent and timing of gut coiling, and size at flexion. The identity of *S. punctata* was also confirmed by comparison to reared larvae.

Though clearly separating *Sillaginodes punctata* from *Sillago schomburgkii*, fin meristics and vertebral counts overlap in the other two South Australian sillaginid species (*S. bassensis* and *S. flindersi*), thus making the specific separation of their larvae difficult. Sillaginid larvae from southern Tasmania (where only *S. flindersi* are found) and larvae believed to be *S. flindersi* from New South Wales (NSW) coastal waters were compared to the third sillaginid larval type collected in South Australia in order to ascertain its identity. The NSW and Tasmanian (referred to herein as eastern) specimens were highly similar but differed from the South Australian type with respect to two pigment characters. First, eastern specimens had a single prominent, elongate melanophore located below the level of the pectoral fin base and overlying the cleithrum that was absent in South Australian material (Fig. 1). Second, eastern specimens developed external lateral midline pigment on the tail at an earlier size (7.2 mm) than did South Australian material (14.8 mm). Two sillaginids are known from Tasmanian waters: *Sillaginodes punctata* and *Sillago flindersi* (Last et al., 1983). Only *S. flindersi* is known to spawn in Tasmanian waters. The eastern form was thus identified as *S. flindersi* and the South Australian specimens as *S. bassensis*. Insufficient material was available across the full size range to render an adequate description of the larval development of *S. flindersii,* and thus this species is not treated in further detail here.

The most useful meristic character separating the three South Australian larval types was number of myomeres. *Sillaginodes punctata* with 42–45 myomeres are easily distinguished from *Sillago schomburgkii* with 36–38 and *S. bassensis* with 32–35. Meristic details for these three species and *S. flindersi* are listed in Table 1.

Descriptions

King George whiting (*Sillaginodes punctata* Cuvier 1829), Figure 2

Material examined—75 specimens, 2.0–30.5 mm BL (CSIRO L587-01, L587-02, L587-03, L587-04, L587-05, L587-06; L588-01; L589-01).

Larval development—The pelagic eggs of *S. punctata* are spherical and have an unsegmented yolk and smooth chorion. Late stage eggs are 839–935 microns in diameter (mean 880, n=25) and have a single oil droplet 246–263 microns in diameter (mean 255, n=25). Reared larvae hatched at 2.00–2.15 mm (mean 2.07, n=24) at 16.5–18.7°C. The timing of fertilization was not recorded as spawning occurred in brood stock tanks overnight. Estimates for incubation period are 48–60 hours. The temperature of the spawning tank was 16.5°C and fertilized eggs were transferred to a 90-liter tank held at 18.0–18.7°C for subsequent incubation, 24 hours prior to hatching.

Newly hatched larvae have a posteriorly located oil droplet and adopt a head-down position in rearing containers. Yolk absorption was complete in reared larvae by 3.5 mm (8 days), although the small-

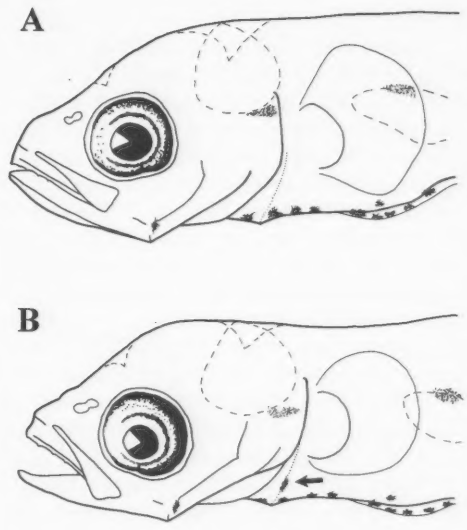

Figure 1

Detail of head and trunk pigment of (**A**) *Sillago bassensis* and (**B**) *Sillago flindersi*. Myomeres have been omitted for clarity. Position of cleithrum is indicated by a dotted line. Arrow indicates characteristic melanophore overlying cleithrum in *S. flindersi*.

Table 1

Selected early life history features useful for identifying sillaginid larvae (sizes are in mm).

Species	Size at gut coiling	Size at flexion	Size at first lat. midline pigment	Size at first dorsal banding	Size at settlement	Completion of fin formation[6]	Number of myomeres	Dorsal fin	Anal fin	Pectoral fin
Sillaginodes punctata[1]	21.0–24.0	5.7–7.0	8.0	6.5–7.0	15.0–18.0	C,P1,A,D2+D1,P2	42–45	XII–XIII+I,25–27	II,21–24	13–15
Sillago bassensis[1]	4.1–7.5	4.8–6.5	14.0	12.0–13.0	12.0–13.0	C,P1,A,D1+D2,P2	32–35	X–XII+I,16–19	II,18–20	15–16
Sillago schomburgkii[1]	>5.1<10.1[7]	4.8-?	2.7	<10.1	12.0–13.0	[8]	36–38	X–XII+I,19–22	II,17–20	15–16
Sillago ciliata[2,3]	<5.0	4.0–5.6	5.3	6.5	15.5	C,A,D2,D1,P1,P2	30–34	XI+I,16–18	II,15–17	15–17
Sillago maculata[2]	[8]	4.6–6.5	3.3	10.6	[8]	C,P1,A,D2,D1,P2	33–36	XI–XII+I,19–21	II,19–20	15–17
Sillago sihama[4]	5.9	5.9	5.9	9.0	[8]	[8]	33–34	XI+I,20–23	II,21–23	15–17
Sillago japonica[5]	<7.6	<7.6	7.6	7.6	11.5	[8]	35–37	XI+I,21–23	II,22–24	15–17

[1] This study.
[2] Miskiewicz, 1987.
[3] Munro, 1945.
[4] Uchida et al., 1958.
[5] Mito, 1966 (as *Sillago japonicus*); Kinoshita, 1988.
[6] Based on all elements present and ossified, C = caudal, P1 = pectoral, P2 = pelvic, A = anal, D1 = first dorsal, D2 = second dorsal.
[7] No specimens between 5.1 and 10.1 mm were available. Coiling of the gut had not commenced in the 5.1-mm specimen but had been completed in the 10.1-mm specimen.
[8] Data not available.

est field-collected larvae (2.9 mm) had already completed yolk absorption.

Larvae are elongate (BDp=11–16% BL) and have 42–45 myomeres (17–21 abdominal + 23 – 27 candal). Body depth at anus increases slightly from 7% to 9% BL during development. Other body proportions remain relatively constant (Table 2). The gut is initially straight and differentiates into defined fore, mid and hind gut sections by 3.7 mm. The gut exhibits some convolution but does not coil during the larval phase. The midgut becomes rugose by approximately 5.0 mm and remains so, although overlying musculature obscures this feature in postsettlement larvae larger than 21.0 mm. The gut begins to coil in postsettlement larvae of 21.0 –24.0 mm and is complete by 26.0 mm. Coiling of the gut proceeds without migration of the anus and is achieved by elongation and anterior looping of the midgut. Consequently, body proportions do not show a significant change in preanal length which remains at 50–52% BL. The gas bladder is first visible in reared larvae by 3.5 mm (5 days) and is prominent and inflated in 86% of field-collected larvae (random subsample, n=50; all larvae collected at night) and all postsettlement larvae collected (all postsettlement larvae collected during day). The gas bladder has its origin at myomeres 2–5 in preflexion larvae but migrates posteriorly during development to myomeres 13–18 by 18.7 mm.

The snout is initially slightly concave in profile, but after flexion, this gradually changes to straight or slightly convex. The eye is round. The mouth initially reaches to below the eye, but is short of the eye in postflexion larvae. Six to eight small villiform teeth are present on the premaxilla by 5.8 mm. The number of teeth increases to 10–12 by late flexion (6.5–7.0 mm). There are no head spines.

Scales are first present around the gut and lateral midline by approximately 27.5 mm.

The development of fins in larval and juvenile *S. punctata* is summarized in Table 1. Completion of fin development occurs in the following sequence: caudal; pectoral; anal and second dorsal (almost simultaneously); first dorsal; and pelvic.

The rays of the caudal fin are present just prior to flexion in larvae of 5.6 mm. Flexion commences by 5.7–6.0 mm and is usually complete by 7.0 mm. Pectoral fin buds are present in reared larvae as slight swellings on the body above the anterior margin of the oil droplet by 3.1 mm (2 days post hatch). Incipient rays are first visible by 7.5 mm and commence ossification by 8.5 mm. A full complement of 13–15 pectoral rays is present by 11.5 mm. Anal and second dorsal fin anlagen appear during flexion (5.8 mm). Distinct bases are present by 7.0 mm, incipient rays by 7.2 mm, and ossification commences by 8.0 mm. The anal and second dorsal fins complete development by 13.0 mm. The

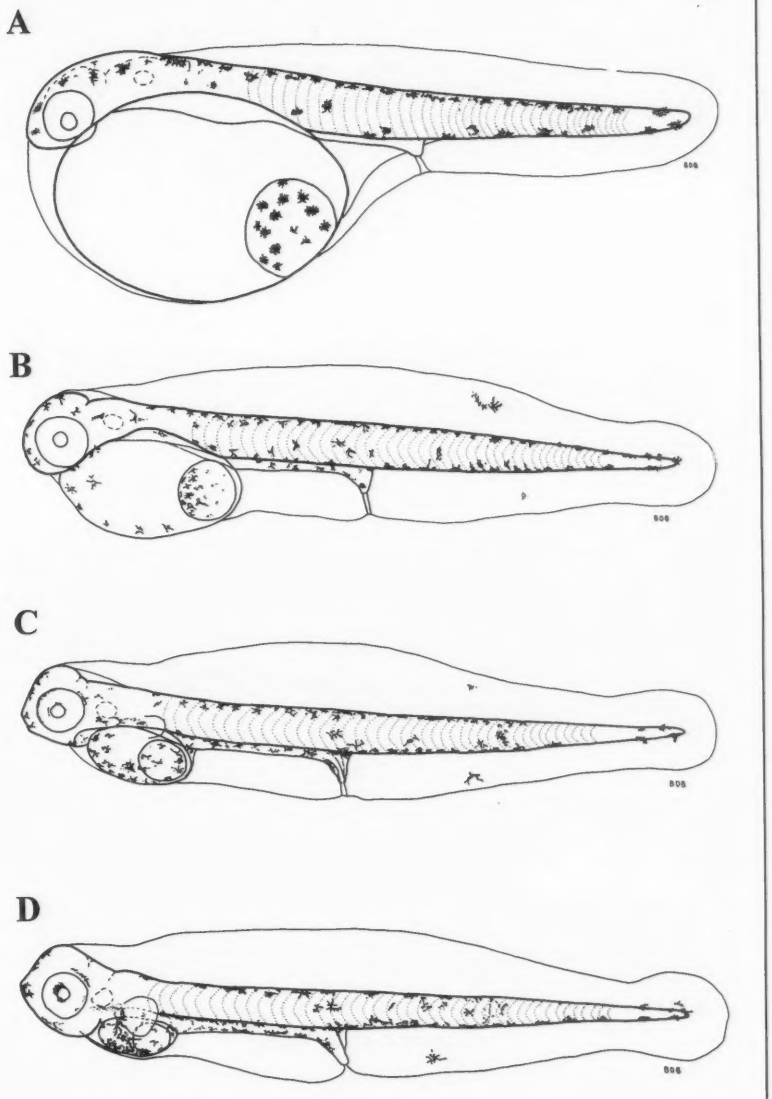

Figure 2

Development of *Sillaginodes punctata*. (**A**) 2.1 mm; (**B**) 2.9 mm; (**C**) 3.1 mm; (**D**) 3.5 mm; (**E**) 3.6 mm; (**F**) 4.2 mm; (**G**) 5.8 mm; (**H**) 6.5 mm; (**I**) 8.5 mm; (**J**) 12.0 mm; (**K**) 18.7 mm postsettlement; (**L**) 22.4 mm (postsettlement: myomeres omitted for clarity). A–I are reared specimens, J–L are field-collected specimens.

first dorsal fin anlage is present by 6.2 mm. Distinct bases are present by 7.6–8.6 mm and ossification of spines has commenced by 8.5–8.9 mm. The first dorsal fin completes development by 13.1 mm. Pelvic fins first appear as slight swellings on either side of the gut in 9.2-mm larvae. Well-developed buds are present by 13.0 mm, incipient rays form shortly thereafter. The pelvic fin does not complete development until 20.0–21.5 mm.

Larval pigment—The oil droplet is well pigmented with large stellate melanophores from at least 24

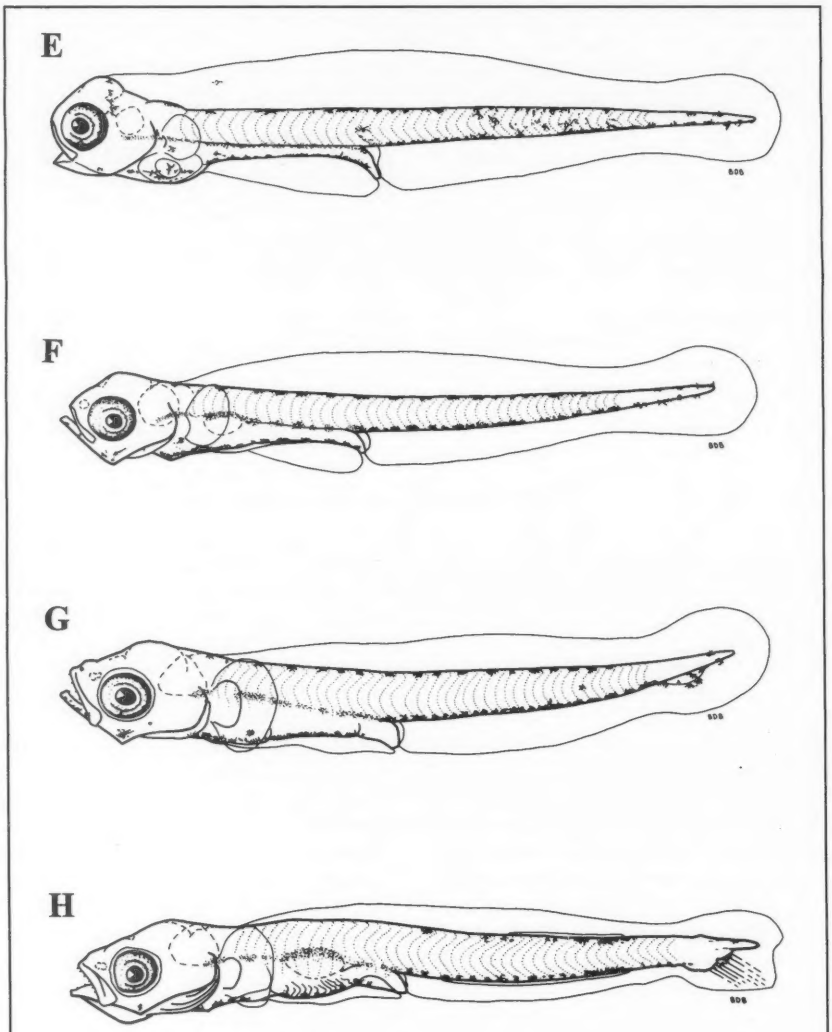

Figure 2 (continued)

hours prior to hatching until yolk exhaustion. Newly hatched larvae have melanophores scattered over the body. Melanophores appear on the ventral and anterior regions of the yolk sac by 2.8 mm and pigment also appears within the finfold (both dorsal and anal) between myomeres 25–32 in reared larvae (not apparent in field-collected larvae — probably owing to finfold damage). Finfold pigment disappears by 3.5 mm.

Initially, melanophores are scattered over the snout but they disappear by 3.5 mm. Pigment appears at the angle of the lower jaw and is retained throughout the larval period. Melanophores are typically present on the lower jaw, ventrally on the gular membrane, and internally below the otic capsule. Further pigment does not form on the head until after settlement.

The dorsal surface of both the gut and the gas bladder are covered with melanophores during development. A linear series of discrete melanophores is present on the ventral midline of the gut in preflexion and flexion larvae. Ventral melanophores disappear from the hindgut by 10.0 mm and this region then remains unpigmented. Concurrently, the remaining 5–8 melanophores between the cleithral symphysis and the hindgut become elongate and are retained in postsettlement larvae.

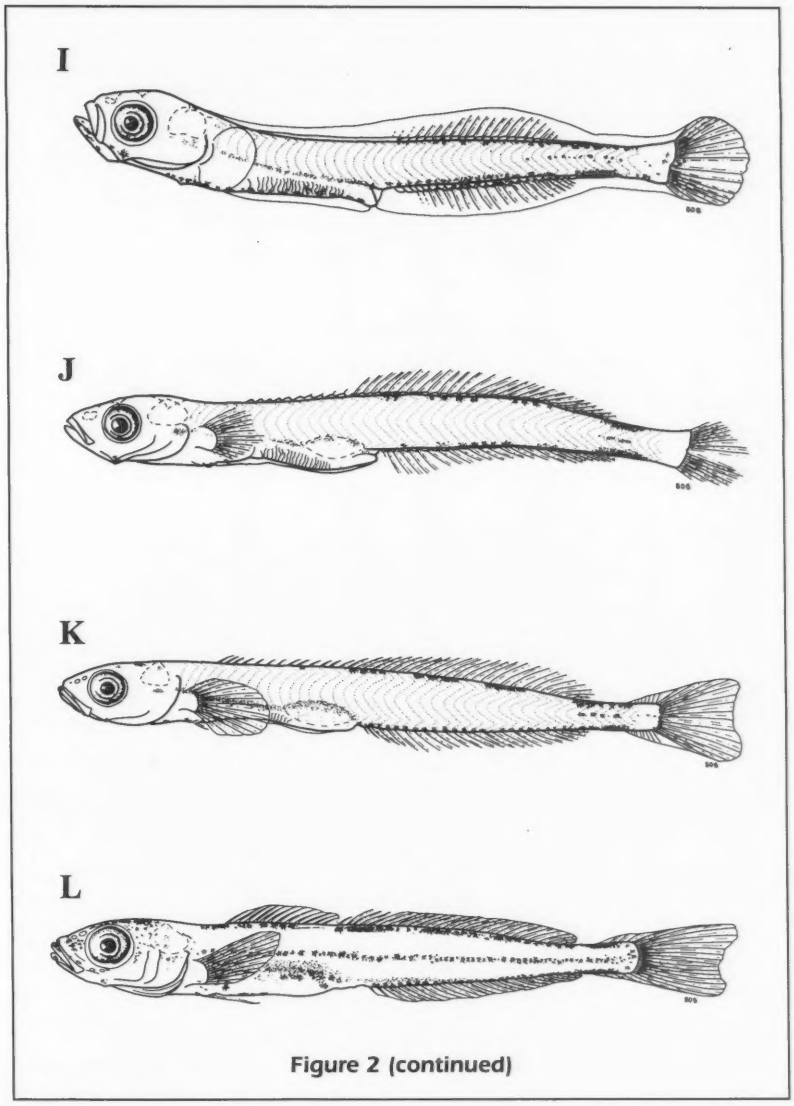

Figure 2 (continued)

By 4.0 mm, pigment on the dorsal surface of the trunk and tail coalesce to form 11–18 discrete, evenly placed melanophores that extend in a linear series posteriorly from the nape to within about 4 or 5 myomeres from the notochord tip. The dorsal surface of the notochord tip has 0–3 melanophores (most commonly 1 or 2) and when present they are useful in separating preflexion *Sillaginodes punctata* from *Sillago bassensis* and *S. schomburgkii*, both of which lack pigment dorsally on the notochord tip. The dorsal series of melanophores on the trunk and tail gradually disappears by the end of flexion (6.5–7.0 mm), excepting those between myomeres 31–40, which become prominent and may extend laterally over the body surface when expanded. Lateral midline pigment develops in this area during late flexion and is retained throughout the postflexion stage. Dorsal pigment gradually redevelops in postflexion larvae as a series of discrete bands, each comprising 3 or 4 pairs of stellate melanophores. Postsettlement larvae have 4–6 such bands which subsequently increase in number to 8–10 as juvenile pigmentation develops.

Ventral pigment on the tail in newly hatched larvae is initially scattered but coalesces to form a se-

Table 2

Body proportions of larvae of *Sillaginodes punctata* (expressed as a percentage of body length). d = damaged; g = gas bladder not visible; — = character not yet formed. Specimens between dotted lines were undergoing flexion.

Body length (mm)	Pre-anal length	Pre-dorsal fin length	Pre-gas-bladder length	Head length	Snout length	Eye diameter	Vent to anal fin length	Body depth at pectoral	Body depth at anus
3.1	51.6	—	37.1	22.5	6.4	9.7	—	12.9	8.1
3.2	53.1	—	23.4	18.7	3.1	6.2	—	10.9	6.2
3.3	51.5	—	28.8	24.2	6.1	9.1	—	15.1	9.1
3.6	51.3	—	22.2	19.4	4.1	8.3	—	11.1	5.5
3.7	51.3	—	24.3	21.6	4.1	8.1	—	12.2	6.8
4.1	47.5	—	24.4	19.5	3.7	7.3	—	12.2	6.1
4.2	50.0	—	23.8	19.0	2.3	7.1	—	11.9	7.1
4.3	55.8	—	32.5	23.2	4.6	9.3	—	16.2	9.3
4.7	51.1	—	34.0	23.4	4.2	8.5	—	14.9	8.5
5.0	50.0	—	30.0	24.0	5.0	8.0	—	13.0	8.0
5.3	52.8	—	32.1	22.6	3.7	7.5	—	13.2	8.5
5.4	50.0	—	29.6	20.4	1.8	7.4	—	13.0	7.4
5.7	47.3	—	31.6	22.8	5.3	7.0	—	12.3	7.9
5.9	45.8	—	28.8	18.6	5.1	6.8	—	11.9	6.8
6.0	48.3	60.0	31.6	23.3	6.7	6.7	—	13.3	8.3
6.2	50.0	—	31.4	21.0	4.0	6.4	6.4	12.1	8.1
6.3	47.6	50.8	33.3	23.8	6.3	6.3	4.2	11.1	7.9
6.4	51.6	46.9	32.0	20.3	6.2	6.2	—	12.5	7.8
6.5	47.7	49.2	33.1	23.8	6.1	d	6.9	12.3	7.7
6.6	47.0	—	30.3	22.7	5.3	6.1	6.6	10.6	7.5
6.8	50.0	48.5	30.9	20.6	5.9	6.6	—	12.5	8.1
7.0	51.4	52.3	34.3	21.4	d	d	—	11.4	10.0
7.2	54.1	50.0	37.5	23.6	5.7	7.6	0.7	11.1	8.3
7.6	52.6	53.9	34.2	22.3	5.3	7.2	2.6	11.8	7.9
8.4	52.3	40.4	40.4	21.4	5.9	7.1	0.0	12.5	10.7
9.3	52.3	30.1	39.7	21.5	6.4	6.4	1.6	10.7	9.1
10.3	52.4	31.1	40.8	21.3	5.8	6.8	0.5	11.1	9.7
12.0	50.0	27.5	39.2	21.7	6.7	5.0	0.6	10.0	9.2
13.1	49.6	27.5	40.4	19.8	5.3	5.3	0.7	9.2	8.4
15.7	50.9	27.4	40.8	19.1	5.7	d	0.0	10.2	11.5
16.1	50.9	26.7	41.0	19.2	5.0	5.6	0.0	9.9	9.9
18.2	51.1	27.5	40.6	20.3	6.0	6.0	0.5	9.9	10.4
18.7	49.2	25.7	38.0	19.8	5.3	5.9	1.1	9.6	9.1

ries of closely spaced melanophores extending to the notochord tip by 3.6 mm. Preflexion larvae have 2–4 melanophores ventrally on the notochord tip. During flexion, melanophores between myomeres 23–38 become more prominent (similar to the dorsal series). The ventral series of melanophores on the tail becomes gradually obscured by overlying musculature (excepting the prominent region between myomeres 32–38) in postflexion larvae. Paired external melanophores develop ventrally on the tail in larvae greater than 8.5 mm and by settlement stage, approximately one pair per myomere is present. This ventral series forms a regular pattern of expanded and contracted melanophores in postsettlement specimens, matching the banding pattern of the dorsal series.

School whiting (*Sillago bassensis* Cuvier, 1829), Figure 3

Materials examined—40 specimens, 2.3–17.2 mm BL (CSIRO L586-01—10 specimens).

Larval development—The smallest *S. bassensis* larva examined was 2.3 mm BL. At this size the mouth and gut are functional, the eyes are pigmented, a gas bladder is present, and yolk absorption is complete.

Larvae are elongate (BDp=13–20% BL) and have 32–35 myomeres (11–15+19–23). Body depth at anus increases slightly from 8–12% BL during development. Other body proportions remain relatively constant (Table 3). The gut forms a convoluted tube in the smallest specimen and is already differentiated

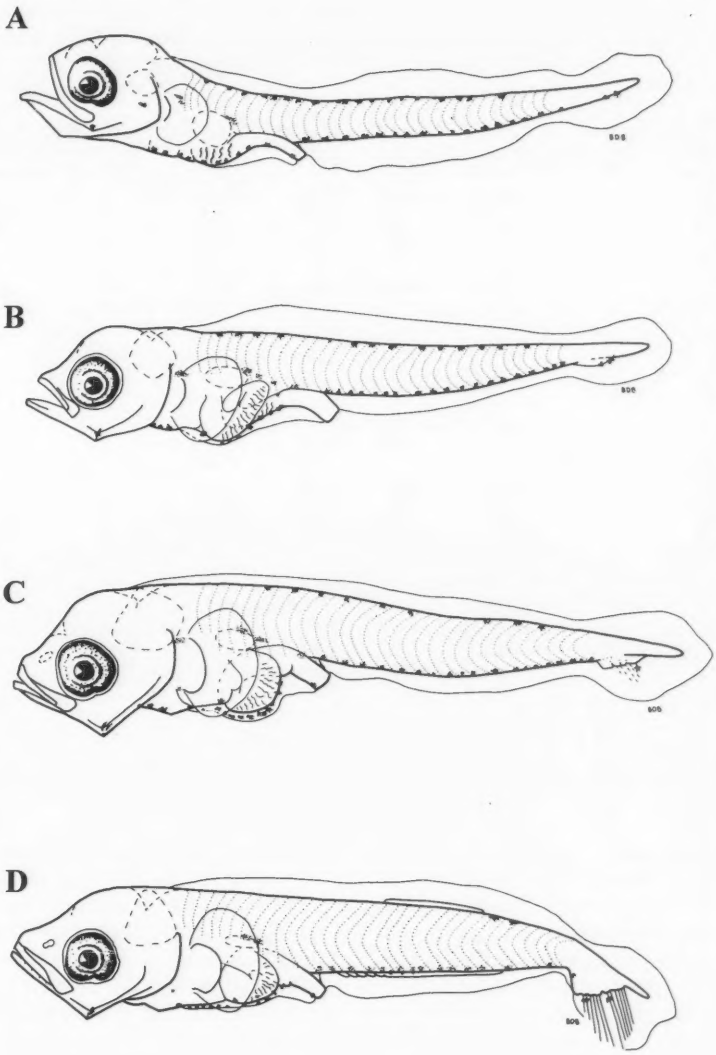

Figure 3

Development of *Sillago bassensis*. (**A**) 3.2 mm; (**B**) 4.4 mm; (**C**) 4.8 mm; (**D**) 5.9 mm; (**E**) 7.2 mm; (**F**) 11.2 mm; (**G**) 12.7 mm (postsettlement).

into fore, mid, and hindgut regions. The midgut becomes rugose by 3.0 mm and remains so, although overlying musculature obscures this feature prior to settlement. The gut begins to coil in preflexion larvae by 4.1 mm. Coiling proceeds without migration of the anus and is achieved by elongation and anterior looping of the midgut (Fig. 4). Consequently, body proportions do not show a significant change in preanal length which remains at 47–48% BL. Coiling of the gut is completed in postflexion larvae (7.0–7.5 mm). The gas bladder has its origin at myomeres 2–8 in preflexion larvae but migrates posteriorly during development to myomeres 5–10 in postflexion larvae. The gas bladder is inflated and prominent in 90% of field-collected larvae (random subsample n=40; all larvae were collected at night).

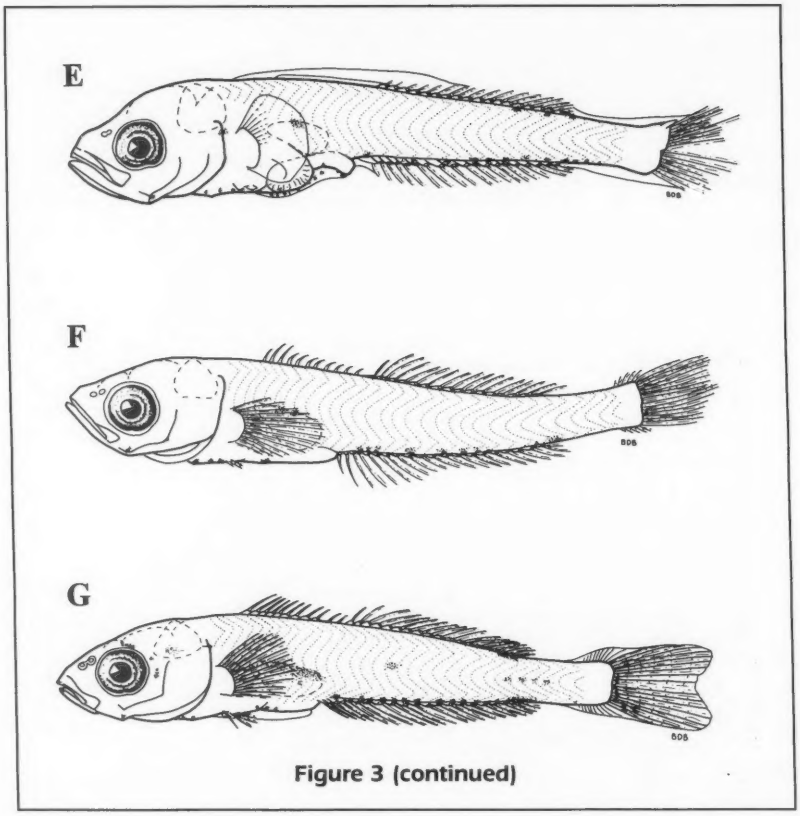

Figure 3 (continued)

The snout is initially slightly concave in profile, but after flexion, this gradually changes to straight or slightly convex. The eye is round. The mouth initially reaches to below the center of the eye but extends only to the anterior margin of the eye in postflexion larvae. Four to six small villiform teeth are present on the premaxilla by 4.7 mm. The number of teeth increases to 7 or 8 during flexion (4.8–6.5 mm). Head spination is only weakly developed. A single minute preopercular spine is present by 7.8 mm but is not visible after settlement (12.5 mm). A weak posttemporal ridge is present by 7.2 mm and is retained; however, no posttemporal spines develop. The single opercular spine is first visible by 12.7 mm and is retained in juveniles.

Scales develop after settlement and are first visible around the gut and lateral midline of the tail by approximately 16.0 mm.

The development of fins in larval and juvenile *S. bassensis* is summarized in Table 1. Completion of fin development occurs in the following sequence: caudal; pectoral; anal, first dorsal and second dorsal fins (almost simultaneously); and pelvic.

The rays of the caudal fin are present just prior to flexion in larvae of 4.4 mm. Flexion commences by 4.8–5.0 mm and is complete by 6.8 mm. Pectoral fin buds are present in the smallest specimen (2.3 mm), incipient rays form during flexion (5.8–6.0 mm), and a full complement of 15 or 16 rays is present by 10.0 mm. Anal-fin and second-dorsal-fin anlagen appear during flexion. Distinct bases are present by 6.5–7.0 mm, incipient rays by 6.8–7.1 mm, and ossification of posterior rays has regularly commenced by 7.2 mm. Ossification of dorsal and anal elements proceeds anteriorly and both fins complete development by 10.0–10.5 mm. The first dorsal fin anlage is present by 7.0 mm. Distinct bases are present by 7.5–8.0 mm and ossification of spines has commenced by 8.0–8.5 mm. Development of the first dorsal fin is complete by 10.0–10.5 mm. Pelvic fin buds are present in 7.0–7.2 mm larvae below the pectoral fin bases. Development of the pelvic fin is complete by 12.5 mm.

Larval pigment—*S. bassensis* larvae were the least pigmented of the three sillaginid species examined.

Pigment on the head in preflexion larvae is limited to the angle of the lower jaw and internally to

Table 3

Body proportions of larvae of *Sillago bassensis* (expressed as a percentage of body length). d = damaged; g = gas bladder not visible; — = character not yet formed. Specimens between dotted lines were undergoing flexion.

Body-length (mm)	Pre-anal length	Pre-dorsal fin length	Pre-gas-bladder length	Head length	Snout length	Eye diameter	Vent to anal fin length	Body depth at pectoral	Body depth at anus
2.3	56.5	—	g	23.9	4.3	8.6	—	17.4	6.5
3.1	48.4	—	27.4	24.2	d	d	—	16.1	8.1
3.4	44.1	—	23.5	19.1	5.9	7.3	—	14.7	5.9
3.7	51.3	—	28.4	d	4.1	8.1	—	16.2	8.1
3.8	47.4	—	25.0	22.4	3.9	9.2	—	17.1	6.6
4.1	51.2	—	29.3	26.8	6.1	8.5	—	18.3	8.5
4.2	52.4	—	29.8	26.2	7.1	8.3	—	19.0	10.7
4.3	46.5	—	g	23.2	5.8	7.0	—	13.9	6.9
4.4	52.3	—	34.1	28.4	6.8	9.1	—	20.4	11.4
4.5	50.0	—	g	24.4	4.4	7.7	—	15.5	8.9
4.6	44.6	—	28.2	23.9	6.5	8.7	—	14.1	7.6
4.7	44.7	—	29.8	22.3	6.4	7.4	—	12.8	6.4
4.8	54.2	—	36.4	29.2	6.2	8.3	2.1	18.7	12.5
4.9	44.9	—	29.6	22.4	5.1	8.2	—	13.3	8.2
5.3	47.2	—	33.0	26.4	7.5	7.5	—	15.1	8.5
5.5	50.9	38.1	g	30.9	9.1	9.1	0.0	20.9	12.7
5.7	50.9	—	31.6	29.8	8.8	8.8	0.0	17.5	10.5
5.8	44.8	56.9	56.9	25.9	6.9	7.7	0.6	13.8	7.7
5.9	49.1	55.9	g	27.1	8.5	8.5	2.5	18.6	11.0
6.3	46.0	47.6	33.3	26.2	6.3	7.9	0.8	14.	8.7
7.7	49.3	54.5	32.5	25.3	7.8	7.8	0.0	18.8	13.6
7.9	45.6	53.2	32.9	25.3	6.3	7.6	2.5	13.9	8.9
8.9	51.7	38.2	34.3	28.6	7.8	7.8	0.0	18.5	16.3
9.6	47.9	33.3	37.5	25.0	7.3	8.3	0.0	14.6	12.5
10.1	44.5	30.7	g	22.8	4.9	7.9	0.9	14.8	10.9
10.2	45.1	31.3	g	24.5	5.9	8.3	1.0	14.7	10.8
11.3	46.9	33.6	33.6	28.3	7.1	8.0	0.0	16.8	13.3

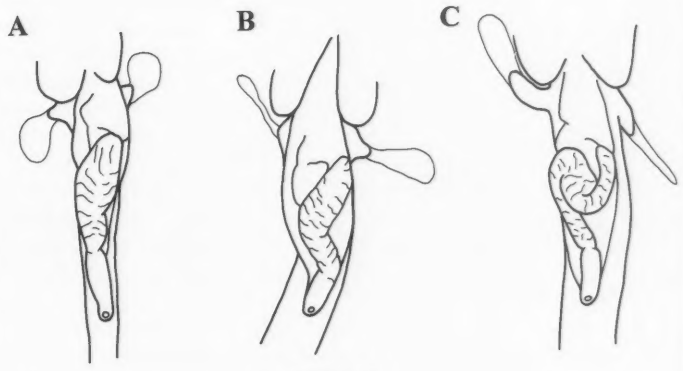

Figure 4

Morphology of the gut in *Sillago bassensis* (ventral view of pigment omitted). (**A**) 2.9 mm; (**B**) 4.2 mm; (**C**) 5.5 mm.

below the otic capsule. Melanophores are irregularly present ventrally on the gular membrane. Additional pigment on the head does not develop until after settlement. Melanophores then develop immediately anterior to and above the eye as well as on the snout and lower jaw. Larger specimens quickly develop a cap of melanophores over the mid and hindbrain.

Pigment on the dorsal surface of the gut consists of 2–7 approximately evenly spaced melanophores in preflexion larvae. This reduces to 2 or 3 just prior to flexion. In postflexion larvae, internal pigment over the gut is restricted to above the gas bladder. Ventral pigment on the gut consists of a midline series of 8–14 melanophores extending from just anterior to the cleithral symphysis to the anus in both preflexion and flexion larvae. One to two additional melanophores are usually present either side of this series below the level of the pectoral fin base (76% of larvae, random subsample n=25), forming a diamond pattern when viewed ventrally (Fig. 5).

Preflexion larvae have 10–18 discrete, evenly placed melanophores that extend in a dorsal linear series on the trunk and tail to within 1–3 myomeres of the notochord tip. The dorsal surface of the notochord tip remains unpigmented throughout development. The dorsal series of melanophores gradually disappears during flexion (4.9–6.5 mm). Postflexion larvae have 0–3 melanophores (most commonly 0) below the bases of the second dorsal fin. Dorsal pigment redevelops after settlement as a series of discrete bands each comprising 3–6 pairs of stellate melanophores. The first of these bands develops immediately below the posterior-most second dorsal fin rays, 5 or 6 additional bands subsequently develop anteriorly, and a single band developes posteriorly on the caudal peduncle by 20.0 mm. Lateral midline pigment on the tail does not form until after settlement, although some internal pigment may be present over vertebrae between myomeres 25–30 after 11.0 mm.

A single row of 14–19 melanophores is present along the ventral midline of the tail in preflexion larvae. This ventral row is gradually obscured by overlying musculature during flexion. Paired external melanophores subsequently develop ventrally on the tail in postflexion larvae, approximately one pair per myomere. After settlement, this ventral series forms a regular pattern of expanded and contracted melanophores producing a similar banding pattern to the dorsal series. One to two (most commonly 2) melanophores are present ventrally on the notochord tip in preflexion larvae. These are retained in postflexion larvae and, with additional melanophores, form a band of pigment over the caudal-fin ray bases.

Yellow fin whiting (*Sillago schomburgkii* Peters 1865), Figure 6

Material examined—16 specimens, 2.7–18.7 mm BL.

Larval development—The smallest *S. schomburgkii* examined was 2.7 mm. At this size the mouth and gut are functional, the eyes are pigmented, a gas bladder is present, and yolk absorption is complete.

Larvae are elongate (BDp=14–18% BL) and have 36–38 myomeres (15–17+20–22). Body depth at anus increases from 8 to 16% BL during development. Other body proportions remain relatively constant (Table 4). The gut forms a convoluted tube in the smallest specimen and is already differentiated into fore, mid and hindgut regions. The midgut becomes rugose by 4.4 mm and remains so, although overlying musculature obscures this feature prior to settlement. The gut has not begun coiling in the largest flexion-stage larva available (5.1 mm). Coiling of the midgut has begun in the 10.1-mm larva and is well developed in all postsettlement larvae. Insufficient specimens were available to further document the timing of gut coiling. Coiling of the gut proceeds without migration of the anus and is achieved by elongation and anterior looping of the midgut. Consequently, body proportions do not show a significant change in preanal length which remains at 51–53% BL. The gas bladder has its origin at myomeres 1–8 in preflexion larvae and is inflated and prominent in all larvae collected during night tows. The gas bladder is inconspicuous in larvae caught during the day.

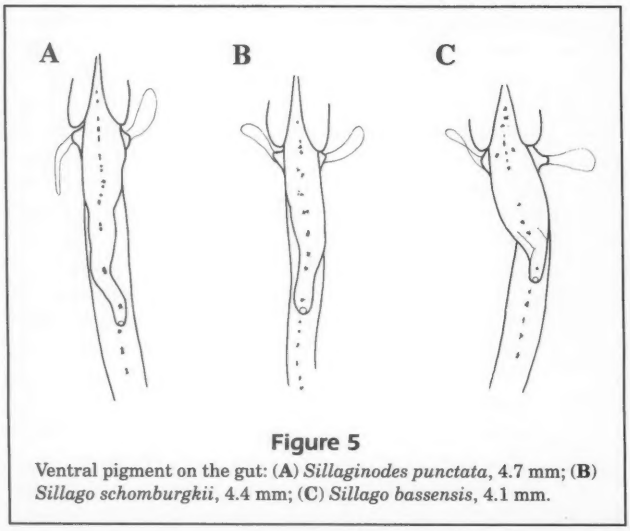

Figure 5

Ventral pigment on the gut: (**A**) *Sillaginodes punctata*, 4.7 mm; (**B**) *Sillago schomburgkii*, 4.4 mm; (**C**) *Sillago bassensis*, 4.1 mm.

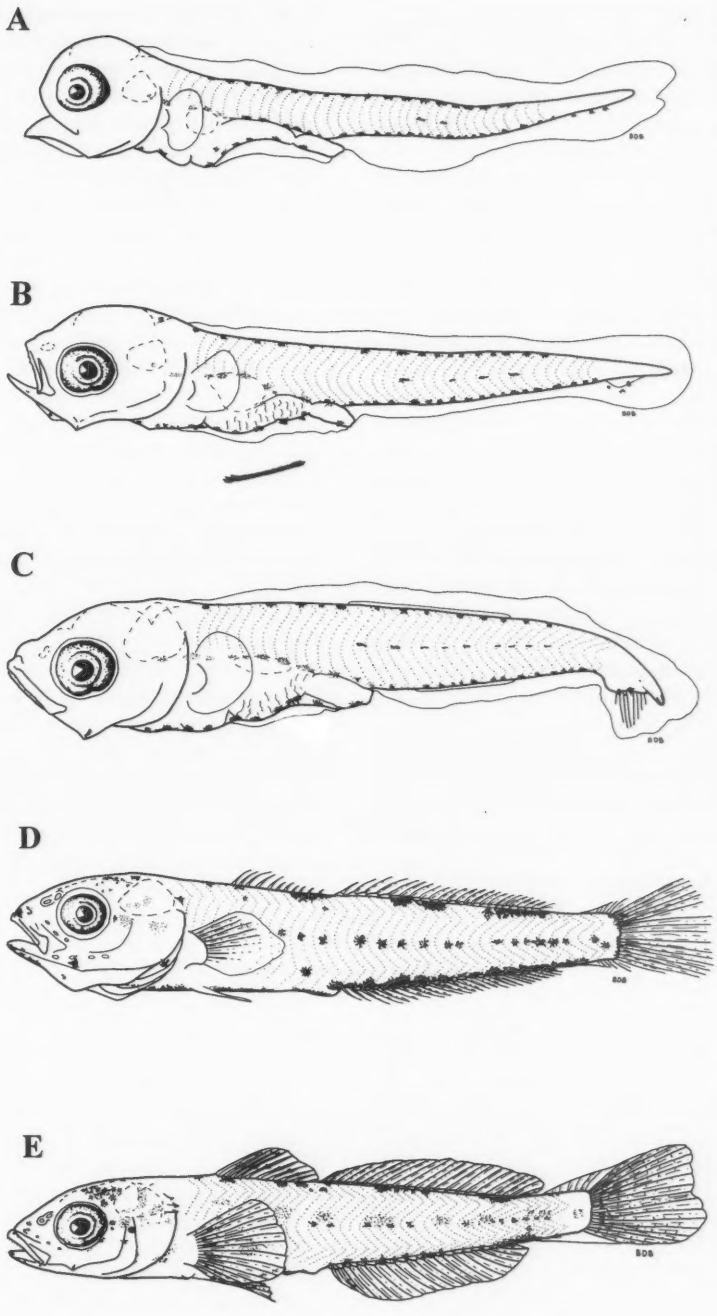

Figure 6

Development of *Sillago schomburgkii*. (**A**) 2.7 mm; (**B**) 4.4 mm; (**C**) 5.0 mm; (**D**) 10.1 mm; (**E**) 13.0 mm (postsettlement).

The snout is initially slightly concave in profile, but after flexion this gradually changes to straight or slightly convex. The eye is round. The mouth initially reaches below the eye but is short of the eye in postflexion larvae. Four to six small villiform teeth are present on the premaxilla by 4.4 mm. The number of teeth increases from 10 to 12 during flexion (from 4.8 to greater than 5.1 mm). Head spination is only weakly developed. One to two preopercular spines are discernible in postsettlement larvae. A weak posttemporal ridge with 1 or 2 small spines is developed in the 10.1-mm postflexion larva and is present in all postsettlement larvae examined. The single opercular spine is not visible in the 10.1-mm larva but is present in postsettlement larvae and is retained in juveniles.

Scales develop after settlement and are first visible around the gut and lateral midline by 17.2 mm.

Insufficient numbers of specimens were available to document the full sequence of fin development or the completion of flexion in *S. schomburgkii*.

The rays of the caudal fin are present in flexion larvae of 4.8 mm. Flexion commences by 4.8 mm. Pectoral fin buds are present in the smallest specimen (2.7 mm) and incipient rays form during flexion. Rays of the pectoral fin have commenced ossification in the 10.1-mm postflexion larva. A full complement of 15 or 16 pectoral fin rays is present in the smallest postsettlement larva (12.7 mm). Anal-fin and second-dorsal-fin anlagen appear during flexion. Full complements (spines and rays) of the anal fin and both dorsal fins are present in the 10.1-mm specimen. The pelvic fin has commenced development in the 10.1-mm larva and has completed development by 12.7 mm.

Larval pigmentation—Pigment on the head in preflexion *S. schomburgkii* larvae is limited to the angle of the lower jaw and internally to the base of the otic capsule. One or two melanophores are also present ventrally on the gular membrane, increasing to three during flexion. Additional melanophores develop on the snout tip, scattered over the lateral surface of the head, and a cap of pigment forms over the mid and hindbrain in postflexion larvae.

Pigment on the dorsal surface of the gut and gas bladder consists of 8–10 approximately evenly spaced melanophores. Scattered internal melanophores gradually spread over the lateral walls of the gut in postflexion larvae. Ventral pigment on the gut consists of a midline series of 8–14 melanophores extending from just anterior of the cleithral symphysis to the anus (Fig. 5).

Preflexion larvae have 15–22 discrete, evenly spaced melanophores that extend in a dorsal linear series from the nape to within 2–5 myomeres of the notochord tip. The number of dorsal melanophores decreases to 13 by 5.0 mm. A series of three discrete dorsal bands consisting of 3–5 paired stellate melanophores has replaced this dorsal series in the 10.1 mm postflexion larva. Lateral midline pigment in *S. schomburgkii* larvae is the most pronounced of all three species examined and is present on the tail in the smallest larva (2.7 mm) as 2 or 3 elongated mel-

Table 4

Body proportions of larvae of *Sillago schomburgkii* (expressed as a percentage of body length). d = damaged; g = gas bladder not visible; — = character not yet formed. Specimens between dotted lines were undergoing flexion.

Body length (mm)	Pre-anal length	Pre-dorsal fin length	Pre-gas-bladder length	Head length	Snout length	Eye diameter	Vent to anal fin length	Body depth at pectoral	Body depth at anus
2.7	53.7	—	24.1	20.4	3.7	9.2	—	14.8	6.3
3.3	51.5	—	25.7	21.2	5.1	9.1	—	15.1	7.6
3.6	48.6	—	g	25.0	6.1	8.3	—	13.9	8.3
3.7	50.0	—	g	24.3	5.4	8.1	—	16.2	9.4
3.8	51.3	—	25.0	22.4	2.6	7.9	—	14.5	7.9
3.9	52.3	—	28.2	22.3	6.4	7.7	—	15.4	7.7
4.0	51.2	—	g	25.0	7.5	7.5	—	17.5	9.0
4.3	52.3	—	g	22.1	5.8	8.1	—	15.1	9.3
4.4	53.4	—	28.4	22.7	5.7	8.4	—	14.8	7.9
4.8	53.1	—	31.2	25.0	6.2	8.3	—	15.6	10.4
5.0	53.0	—	34.0	25.0	7.0	9.0	8.0	18.0	11.0
10.1	52.5	34.6	g	27.7	8.9	6.9	2.0	17.8	15.8
13.6	49.3	32.3	g	27.2	7.3	8.1	3.6	16.9	14.7
17.2	53.5	36.0	g	30.2	9.3	8.1	2.9	17.4	14.5

anophores in the vicinity of myomeres 24–26. Lateral midline pigment spreads both anteriorly and posteriorly as a linear series of elongated myomeres during development. By 10.1 mm, lateral midline pigment consists of 18 stellate and approximately evenly spaced melanophores extending from the pectoral fin to the caudal peduncle. Internal pigment along the vertebrae is visible in the 10.1-mm postflexion larva but is most pronounced in postsettlement larvae as clusters of melanophores located over every 2–5 vertebrae.

A single row of 16–18 melanophores is present along the ventral midline of the tail in preflexion larvae. This ventral row is gradually obscured by overlying musculature during flexion. Paired external melanophores (approximately one pair per myomere) subsequently develop ventrally on the tail in postflexion larvae, approximately one per myomere. Two to three (most commonly three) melanophores are present ventrally on the notochord tip in preflexion larvae. These are retained in postflexion larvae and form a band of pigment over the caudal-fin ray bases.

Discussion

Egg or larval development, or both, have been described for only four other species of sillaginid larvae: *Sillago japonica* (Kamiya, 1925; Ueno and Fujita, 1954; Ueno et al., 1958; Mito, 1966 — as *Sillago japonicus*; Ikeda and Mito, 1988; Kinoshita, 1988; Oozeki et al., 1992); *Sillago sihama* (Gopinath, 1946; Uchida et al., 1958; Ikeda and Mito, 1988; Kinoshita, 1988); *Sillago maculata* (Miskiewicz, 1987; Kinoshita, 1988); and *Sillago ciliata* (Munro, 1945; Miskiewicz 1987; Tosh[4]). In addition, Miskiewicz (1987, p. 62) reported a series of unidentified sillaginid larvae which, based on pigment on the lateral wall of the gut below the pectoral fin base, were almost certainly *Sillago flindersi*.

Characters useful for the identification of tropical sillaginid larvae at the family level and similarity of sillaginid larvae to those from other families have been considered in detail by Leis and Trnski (1989) and Miskiewicz (1987). Although most of the characters discussed by these authors also apply to the temperate species considered here, an exception was the timing of gut coiling. Leis and Trnski (1989) reported that the gut of tropical sillaginid larvae commenced coiling during notochord flexion and was accompanied by the anterior migration of the anus. In the South Australian species, coiling of the gut commenced prior to flexion in *S. bassensis*, after settlement in *Sillaginodes punctata,* and had not yet commenced in the largest flexion larva available for *Sillago schomburgkii* (although coiling of the gut was present in a 10.1-mm postflexion larva). In all cases, coiling of the gut proceeded without migration of the anus and was achieved by anterior looping of the midgut. The implications of these variations are unclear but suggest that, although useful on a specific level, the timing of gut coiling and migration of the anus are not useful characters for the identification of temperate sillaginids at the family level.

The significance of gut coiling may relate to shifts in diet. Robertson (1977) reported a dietary shift in postsettlement *Sillaginodes punctatus* (= *punctata*) in Westernport Bay (Victoria) between November and December, a shift from harpacticoid copepods, gammarid amphipods, and mysids to larvae of the ghost prawn *Callianassa australiensis*, polychaetes, and juvenile crabs. Robertson correlated this dietary shift with increasing body size and mouth gape as well as with the availability of *C. australiensis* larvae. However, from his length-frequency data, this period also corresponds to the size range during which postsettlement *S. punctata* undergo gut coiling. Alternatively, because evacuation rates are believed to decrease after gut coiling (Arthur, 1976, and references within; Young[5]), perceived changes in diet may be confounded by increased food retention times. Stomach contents were not analyzed during this study; they provide a valuable topic for further research.

Despite seasonal sampling over five years, larvae of only three of the four sillaginid species with adult distributions extending to South Australia were located during this study. The lack of *Sillago flindersi* larvae suggests either that this species does not spawn in South Australian waters, that sampling frequency was too course to detect the presence of larvae of this species, or that *S. flindersi* larvae behave differently from other sillaginid species and are less prone to capture (e.g. epibenthic and neustonic).

Sillago flindersi larvae are frequently encountered in similar sampling regimes in coastal waters of eastern Australia[6] and in Tasmanian waters (author's pers. observ.) and thus it seems unlikely that a lack of their larvae in South Australian samples represents an artifact of sampling or that their behavior is fundamentally different from other sillaginid larvae.

[4] Tosh, J. R. 1903. Notes on the habits, development etc. of the common food fishes of Moreton Bay. Queensland Marine Dep.: Marine Biologist's Report.

[5] Young, J. W. CSIRO Div. Fisheries, GPO Box 1538 Hobart, Tasmania, Australia 7001. Personal commun., 1993.

[6] Miskiewicz, A. G. Sydney Water Board, PO Box A53, Sydney South, NSW, Australia 2000. Personal commun., 1993.

South Australian waters represent the western distributional limit of *S. flindersi* in southern Australia (McKay, 1992; Gomon et al., 1994; Kailola et al., 1993). Spawning times for *S. flindersi* vary throughout its range; a summer spawning is recorded for Victorian populations (Hobday and Wankowski[7]). No data are available on either the reproductive condition of *S. flindersi* or on the presence or absence of juveniles in South Australian waters. However, on the basis of a lack of larvae, I suggest that spawning does not occur in South Australia and that the western limit of *S. flindersi* comprises fish recruited from eastern populations.

Acknowlegments

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Responses of Tanner crabs, *Chionoecetes bairdi*, exposed to cold air

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Abstract.—Female and sublegal-size male Tanner crabs, *Chionoecetes bairdi*, are often caught incidentally in the males-only fishery for this species. Effects of low air temperature during the winter fishery on juvenile and female adult crabs and on the developing eggs brooded by the females were simulated in the laboratory by exposing crabs to cold air (–20 to +5°C) up to 32 minutes; controls were not exposed. Exposure was expressed as degree·hours (°h), the product of temperature (°C) and time (hours). Severe exposure caused death: median lethal exposure stabilized at –3.3 ± 0.8°h for juveniles and –4.3 ± 0.5°h for adults after 16 days. Exposure also reduced vigor (measured by righting ability), caused pereiopod autotomy, and depressed adult feeding rates and juvenile growth. Exposures causing one-half the crabs to cease righting were –1.2 ± 0.3°h for juveniles and –2.1 ± 0.3°h for adults (measured immediately after exposure). Mean pereiopod autotomy ranged up to 44% for juveniles exposed to –2°h, and up to 10% for adults exposed to –10.6°h. Ecdysis of juveniles was not affected, but exposed juveniles frequently shed additional pereiopods with the molt. Prompt return of incidentally caught Tanner crabs to the sea when temperatures are below freezing should reduce adverse effects of cold aerial exposure.

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Tanner crabs, *Chionoecetes bairdi* Rathbun, 1893, are the target of a large commercial pot fishery and are an important commercial species in Alaskan waters (Otto, 1989). Landings of *C. bairdi* rose to a peak of 57,923 metric tons (t) in 1978, then declined to 5,390 t in 1987; landings increased to 23,507 t in 1990.[1]

Current Alaska fishing regulations require release of small (<139-mm carapace width) male and all female *C. bairdi*. Commercial fishery openings in recent years have generally ranged from November through April,[2] when minimum daily air temperatures can drop to –21°C.[3] The amount of time incidentally captured crabs remain on deck varies, ranging from a few minutes during pot fishing to hours in some trawling operations (Stevens, 1990). Exposure to cold air during fishing operations may be detrimental to individual crabs (Carls and O'Clair, 1990), exposed egg clutches, and possibly—with sufficient fishing pressure—to the population. Regulations also require that Tanner crabs caught incidentally by multispecies trawling operations in the eastern Bering Sea be returned to the sea, but these regulations may be ineffective because of poor survival (22 ± 3.6% for *C. bairdi*) of the culled crabs (Stevens, 1990).

Here we report the responses of juvenile and adult female Tanner crabs and their offspring exposed to cold air. Our objectives were to determine the effects (immediate

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[2] ADF&G (Alaska Department of Fish and Game).
1989a. Report to the Alaska Board of Fisheries. Southeast Alaska and Yakutat (Region 1) 1988/89 shellfish fisheries. Regional Information Rep. No. 1J89-01. ADF&G, Div. Commercial Fisheries, Juneau, AK.
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1989c. Prince William Sound management area shellfish report to the Alaska Board of Fisheries. ADF&G Regional Information Rep. No. 2C89-03. ADF&G, Div. Commercial Fisheries, Central Region, 333 Raspberry Rd., Anchorage, AK 99581, 55 p.
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[3] NOAA. 1987. Local climatological data, monthly and annual summaries with comparative data. U.S. Dep. Commer., National Climatic Data Center, Asheville, NC 28801.

and long-term) of exposure to cold air on 1) survival; 2) sublethal responses, including righting response, limb autotomy, feeding rate, ecdysis (juveniles), and growth; and 3) reproductive responses including egg survival, zoeal production, zoeal viability, and subsequent egg extrusion and viability of the extruded clutch.

Methods

Experimental crabs were collected with crab pots. Juvenile crabs (both sexes) were collected in Auke Bay, Alaska (lat. 58°21'N, long. 134°41'W) on 14 and 19 January 1988. Ovigerous females were captured near Eagle River (lat. 58°31'N, long. 134°48'W) and Lena Point (lat. 58°24'N, long. 134°47'W) in Favorite Channel, Alaska, on 11 February 1988.

In the laboratory, carapace length (distance from the posterior margin of the right ocular orbit to the midpoint of the posterior margin of the carapace) was measured to the nearest millimeter. Carapace width was subsequently estimated by regressing carapace widths and lengths of Tanner crabs measured at a later date.[4] Live weight was measured to the nearest 0.1 g. Juvenile crab weights ranged from 26 to 229 g ($\bar{x}$=109 ±14 g), and carapace lengths ranged from 35 to 64 mm ($\bar{x}$=49 ±2.3 mm) (Fig. 1). Estimated juvenile carapace widths (for both sexes) ranged from 46 to 74 mm (width=–0.237 + 1.318 × length, r^2=0.994, n=145). The immature condition of males was determined solely by body size. Adult female crab weights ranged from 182 to 553 g ($\bar{x}$ = 329 ± 8 g), and carapace lengths ranged from 65 to 96 mm ($\bar{x}$=80 ±1.0 mm) (Fig. 1). Estimated female carapace widths ranged from 85 to 124 mm (width=1.746 × 1.274 × length, r^2=0.995, n=70).

Crabs were maintained in 500-L tanks at ambient seawater temperatures (6.0–6.9°C for juveniles, 5.3–6.0°C for adults) until exposure to test air temperatures; after exposure they were returned to the same tanks for 32–35 days of observation (4.7–6.7°C for juveniles, 4.7–5.2°C for adults). A subset of 40 female crabs was retained for an additional three months of observation.

Crabs were exposed in a modified chest freezer divided by a vertical baffle into two compartments of unequal size (Carls and O'Clair, 1990). Infrared heat lamps were placed in the smaller compartment for temperature control. To ensure uniform temperatures, a small fan (in the center bottom of the baffle) drew air from the exposure chamber into the small chamber at 45 ± 5 cm/sec. Return air circulated over the baffle into the exposure chamber. Temperatures were measured with a thermistor located in the exposure area near the fan and were regulated manually by switching the heat lamps on or off. Temperatures were controlled to ±0.1°C after the chamber had cooled to the desired temperature. Crabs were exposed to cold air on the plywood bottom of the exposure chamber.

Juvenile crabs were randomly placed in six groups with 10 crabs per group and were exposed to cold air on 21 and 25 January (about one week after capture). Exposure temperatures ranged from –5.0 to –20.0°C; exposure durations were 0, 12, 16, or 24 minutes to yield 0, –1.0, –1.5, –2.1, –4.0, and –8.0°h exposures (Table 1). The lengths ($F_{5,54}$=0.06, P>0.99) and

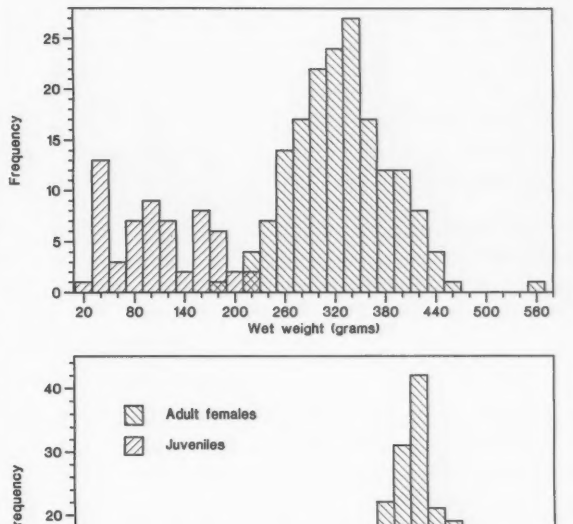

Figure 1

Chionoecetes bairdi length and weight frequencies. Adult female frequencies may not be directly comparable to juvenile frequencies because they were taken from a different locality.

[4] r^2>0.99. Stone, R. NMFS Auke Bay Lab., Juneau, AK 99801–8626. Unpubl. data, May 1992.

Table 1

Temperature and duration of exposure of *Chionoecetes bairdi* to cold air. The number of crabs exposed (*n*) is also indicated. Controls were not exposed to air. SE = standard error.

Air temperature (Celcius) mean	SE	Exposure time (minutes)	Degree-hours	*n*
Juveniles				
—	—	0	0.00	10
–5.0	0.02	12	–0.99	10
–7.5	0.05	12	–1.50	10
–10.2	0.24	12	–2.05	10
–15.0	0.03	16	–4.00	10
–20.0	0.07	24	–8.02	10
Adults				
5.1	0.12	8	0.683	7
5.0	0.01	32	2.672	7
—	—	0	0.000	31
–3.2	0.21	4	–0.211	8
–3.1	0.04	8	–0.411	8
–3.1	0.06	16	–0.813	8
–3.0	0.03	32	–1.621	8
–8.2	0.19	4	–0.544	8
–8.1	0.11	8	–1.075	8
–8.1	0.06	16	–2.149	8
–8.1	0.03	32	–4.299	7
–13.1	0.18	4	–0.875	8
–12.9	0.08	8	–1.720	8
–13.0	0.03	16	–3.472	7
–13.0	0.02	32	–6.933	8
–20.3	0.34	4	–1.353	8
–20.1	0.15	8	–2.676	8
–18.4	0.08	16	–4.899	8
–19.9	0.04	32	–10.597	8

weights ($F_{5,54}$=0.02, P>0.99) of the crabs did not differ significantly between treatments. Change in juvenile crab body weight was estimated from initial and final measurements (32 d).

Female crabs were randomly placed in 20 groups (including controls) in a complete 4 (temperature) by 5 (length of exposure) design, with 7 to 8 crabs per group. Treatment temperatures ranged from –3.1 to –20.3°C and exposure duration ranged from 0 (controls) to 32 minutes (Table 1). Two additional groups were tested at 5°C for 8 and 32 minutes (Table 1). The crabs did not differ significantly in length ($F_{21,149}$=1.13, P=0.324) or weight ($F_{21,149}$=1.36, P=0.149) between treatments. Exposure took place 16 and 17 February (about six days after capture). Observation continued through 22 June.

Mortality and limb autotomy were monitored daily. Crabs were judged dead when scaphognathite movement stopped. Generally, dead crabs were rechecked the following day before they were removed from test tanks. The number of legs missing on each crab was counted and autotomized legs were removed from the tanks.

Righting response (the time it took a crab to right itself when placed on its back underwater), which we considered to be a measure of vigor, was timed to the nearest 0.01 second immediately after aerial exposure and 1, 2, 4, 8, 16, 24, and 32 days thereafter. Crabs that could not right themselves after 2 minutes were recorded as "not righting" and were placed upright in the tank.

A subset of 40 female crabs randomly selected from the entire exposure range was used for reproductive observations. The crabs were isolated 32 days after exposure in covered 70-L tanks that overflowed into 19-L buckets containing conical 363-µ mesh nets designed to trap zoeae. Flow rates were approximately 1.5 L/minute; 95% turnover time was 2.3 hours and water temperatures ranged from 5.2 to 5.9°C during this period (23 March–11 May).

Feeding rates were measured before and after the zoeal hatch while the 40 ovigerous females were individually isolated. Mussels, *Mytilus trossulus,* were fed ad libitum to crabs during each feeding period. Live mussels were cut in half and drained tissue-side down on paper towels for five minutes, weighed, then placed in the tanks. Twenty-four hours later the remaining food was removed, drained, and weighed as before. At each feeding, four food portions were placed as controls in tanks without crabs. Consumption was corrected for the mean weight changes in the control portions. Feeding observations were repeated every 1 to 3 days, from 41 to 60 and from 85 to 98 days after exposure.

Zoeae were collected daily, rinsed from the nets, concentrated in a known volume, and subsampled with a 5- or 10-mL Hensen-Stemple pipette (Carls and O'Clair, 1990). Subsamples, which contained a minimum of 200 zoeae, were preserved in 5% formalin and counted later; the occasional large subsample was divided with a Folsom plankton splitter before being counted. After zoeal hatching, all debris from each tank bottom was preserved to determine the number of dead eggs and zoeae.

Responses of the crabs were related to aerial exposure, expressed as the product of air temperature (°C) and length of time in air (hours), i.e. degree-hours (°h). In a similar experiment, Carls and O'Clair (1990) demonstrated the usefulness of this technique for interpreting responses to aerial exposure in adult king crabs, *Paralithodes camtschaticus*. Because the responses of the Tanner crabs to exposure (in °h) were similar in form to those of the king crabs over identi-

cal treatment ranges (0–32 minutes, –20 to +5°C; see Results section) and could be described by the same types of simple linear or nonlinear models, we used the same technique here.

Regression techniques and logit analysis were used to relate response variables to exposure (Berkson, 1957; BMDP, 1983). We compared median lethal responses with log-likelihood ratio tests (Fujioka, 1986). Multiple regression was used to test for differences in the slopes of regression lines and to adjust for covariates (Kleinbaum and Kupper, 1983). The relation of selected response variables to one another was tested with parametric correlation. After one-way analysis of variance, comparisons of treatment means were made with Tukey's or Dunnett's a posteriori multiple comparison tests and judged significantly different if $P \leq 0.05$. Proportional data were arcsine transformed. Reported error ranges are ±95% confidence limits.

Results

Mortality

Below –1 to –3 degree hours, exposure to cold air killed crabs. Almost all mortality occurred 1–2 days after exposure; in groups where more than half the crabs died, mortality always reached 50% within 2 days. Mortality was inversely related to exposure and increased rapidly below –1°h for juveniles and below –3°h for adults (logistic regressions [large P-values indicate good fits], $P_{juvenile}=0.959$, $P_{adult}=0.882$; Fig. 2). Nearly all deaths occurred within 8 days after exposure; no crabs died after day 16. For juveniles, calculated median lethal exposures rose from –7.7 ± 3.4°h 1 day after exposure to –3.3 ± 0.8°h 16 days after exposure, and for adults from –7.2 ± 1.6°h to –4.3 ± 0.5°h over the same time period (Table 2).

Righting response

The speed with which crabs righted themselves when placed on their backs was inversely related to exposure (Fig. 3A). The response was most clearly described by the percentage of crabs not righting within two minutes (logistic regressions, $P=0.799$ [$n=6$] for juveniles; $P=0.978$ [$n=22$] for adults; Fig. 3B). Percentages of crabs not righting increased sharply below –1.0°h for juveniles and below –2.2°h for adults, and crabs ceased righting entirely after exposure to ≤–4.0°h for juveniles and ≤–6.9°h for adults (Fig. 3B). Median exposures causing one-half the crabs to cease righting (EC50) were –1.2 ± 0.3°h for juveniles and –2.1 ± 0.3°h for adults, measured immediately after exposure; values declined to –1.6 ± 0.3°h for juveniles and –3.8 ± 0.5°h for adults measured 32 days after exposure (Table 3). The percentage of crabs unable to right themselves immediately after exposure was significantly correlated with cumulative mortality ($P_{juvenile}=0.003$, $r^2_{juvenile}=0.91$, $n=6$; $P_{adult}<0.001$, $r^2_{adult}=0.67$, $n=22$) and, therefore, could serve as a predictor of death.

Righting times tended to improve (decrease) during the first eight days after exposure, but this recovery was generally not statistically significant.

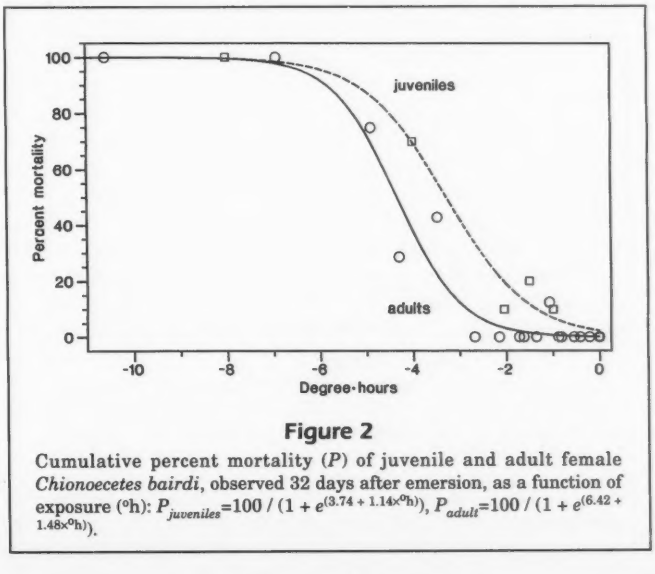

Figure 2

Cumulative percent mortality (P) of juvenile and adult female *Chionoecetes bairdi*, observed 32 days after emersion, as a function of exposure (°h): $P_{juveniles}=100 / (1 + e^{(3.74 + 1.14 \times °h)})$, $P_{adult}=100 / (1 + e^{(6.42 + 1.48 \times °h)})$.

Righting times of juvenile crabs from all exposures tended to decrease over time (Fig. 4). The righting times of adult crabs exposed to ≤–2.2°h generally showed little evidence of recovery. Median exposures causing one-half the crabs to cease righting also generally declined, but 95% confidence bars overlapped.

Pereiopod autotomy

Exposure to cold air caused pereiopod autotomy. Juvenile pereiopod losses increased from 0 to –2°h but declined towards the most severe exposure (–8°h), possibly because early mortality precluded autotomy (Fig. 5A). Juvenile crabs often dropped legs or chela during aerial exposure, but losses also continued after exposure (Fig. 5B), often during ecdysis (Fig. 6).

Adult crabs autotomized fewer pereiopods than juveniles; as with juveniles, loss was most frequent immediately after exposure. Loss of pereiopods in adults was directly related to severity of exposure ($P<0.001$, $r^2=0.85$, $n=19$; Fig. 5A). Autotomy was correlated with mortality in adult crabs ($P<0.001$, $r^2=0.81$, $n=19$) but not in juveniles ($P=0.621$, $r^2=0.07$, $n=6$). Autotomy was also correlated with the percentage of adult crabs not righting as measured immediately after exposure ($P<0.001$, $r^2=0.81$, $n=22$).

Ecdysis

Juvenile crabs began molting 22 January and continued through 21 February. Molt timing was not correlated with exposure ($r^2=0.09$). Juveniles exposed

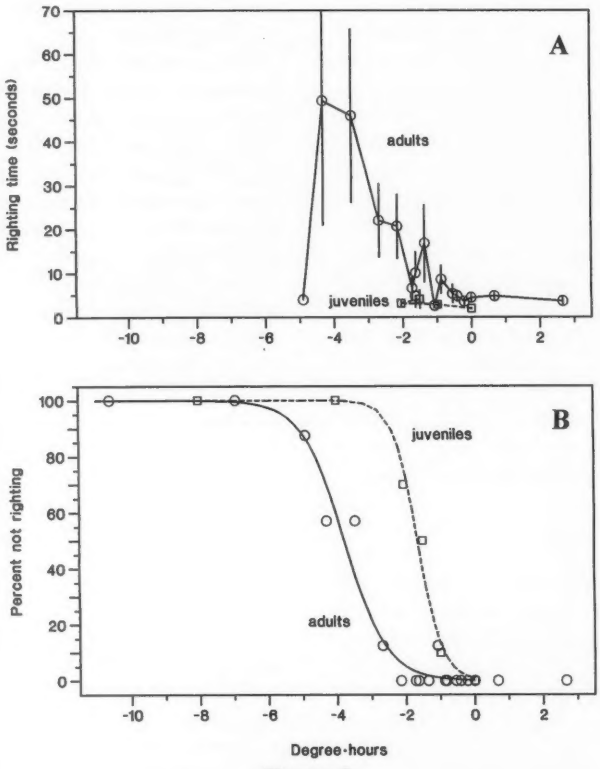

Figure 3

Righting times of juvenile and adult female *Chionoecetes bairdi* capable of righting (**A**), and percentage not righting (**B**) observed 32 days after emersion as functions of exposure (°h). Percentages not righting were $100/(1 + e^{(6.235 + 1.651 \times °h)})$ for adults and $100 / (1 + e^{(4.701 + 2.858 \times °h)})$ for juveniles. Error bars are ±1 standard error. The ability of the single surviving –4.9°h adult crab improved over time; its righting time 32 days after exposure was similar to that of controls.

Table 2

Degree-hours causing death (LC) in *Chionoecetes bairdi* exposed to cold air, estimated with logit analysis. The LC number indicates the percentage of crabs affected; e.g. LC50 is the median lethal degree-hours. The error term (CI) is the estimated 95% confidence interval.

Day	LC10	LC30	LC50	LC70	LC90	CI
Juveniles						
1	−0.9	−5.1	−7.7	−10.4	−14.6	3.4
2	−1.0	−4.6	−6.8	−9.0	−12.6	2.6
4	−1.3	−3.9	−5.5	−7.1	−9.7	1.7
8	−1.3	−2.9	−3.9	−4.8	−6.4	1.1
16	−1.3	−2.5	−3.3	−4.0	−5.2	0.8
32	−1.3	−2.5	−3.3	−4.0	−5.2	0.8
Adults						
1	−3.1	−5.6	−7.2	−8.8	−11.3	1.6
2	−2.8	−5.2	−6.7	−8.2	−10.6	1.5
4	−2.6	−4.8	−6.2	−7.5	−9.7	1.3
8	−3.0	−4.0	−4.6	−5.1	−6.1	0.6
16	−3.2	−3.9	−4.3	−4.8	−5.5	0.5
32	−2.8	−3.8	−4.3	−4.9	−5.8	0.6

Table 3

Effective degree-hours causing cessation of righting (EC) in *Chionoecetes bairdi* exposed to cold air, estimated with logit analysis. The EC number indicates the percentage of crabs affected; EC50 is the median effective degree-hours. The error term (CI) is the estimated 95% confidence interval.

Day	EC10	EC30	EC50	EC70	EC90	CI
Juveniles						
0	−0.2	−0.8	−1.2	−1.6	−2.2	0.3
1	−0.7	−1.2	−1.5	−1.7	−2.2	0.3
2	−0.6	−1.1	−1.5	−1.8	−2.3	0.3
4	−0.5	−1.1	−1.4	−1.7	−2.2	0.3
8	−1.1	−1.4	−1.7	−1.9	−2.3	0.3
16	−0.9	−1.3	−1.6	−1.9	−2.4	0.3
24	−0.8	−1.4	−1.7	−2.1	−2.6	0.3
32	−0.9	−1.3	−1.6	−1.9	−2.4	0.3
Adults						
0	−1.3	−1.8	−2.1	−2.5	−3.0	0.3
1	−2.0	−2.7	−3.1	−3.5	−4.1	0.4
2	−1.8	−2.4	−2.8	−3.2	−3.8	0.4
4	−2.3	−2.8	−3.1	−3.4	−3.9	0.4
8	−2.1	−2.7	−3.0	−3.4	−4.0	0.4
16	−2.2	−2.7	−3.1	−3.5	−4.0	0.4
24	−2.2	−2.9	−3.4	−3.8	−4.6	0.5
32	−2.4	−3.3	−3.8	−4.3	−5.1	0.5

to cold air frequently lost pereiopods during ecdysis; losses increased from 0 to −4°h. The only crab exposed to −8°h that attempted to molt lost no limbs, but died during ecdysis (Fig. 6).

Feeding rates

Feeding rates of adult female Tanner crabs were significantly depressed by exposure to cold air ($P_{ANOVA} \leq 0.001$). In general, adult females exposed to <−2.7°h (62% of the median lethal exposure) ate significantly less than did controls (Tukey test). Feeding rates measured shortly before zoeal hatching (41 to 60 days after exposure) were significantly less ($P \leq 0.05$) for all crabs than feeding rates after zoeal hatching (85–98 days after exposure), but the slopes (feeding rate/exposure) before and after hatching did not differ (multivariate regression, $P > 0.50$; Fig. 7). The frequency of feeding also increased significantly after zoeal hatching ($P < 0.001$) and was significantly related to aerial exposure before and after larval hatching ($P_{linear} < 0.001$). The most severely treated crabs (−4.9°h) did not eat before zoeal release but ate 57% of the time after release.

Weight change

Change in weight of juvenile crabs was reduced by exposure to cold air. Wet weights of juvenile crabs that did not molt declined with increasing exposure severity ($P = 0.002$, $r^2 = 0.42$, $n = 20$; Fig. 8). The weight increment of juvenile crabs that molted also declined with decreasing exposure (Fig. 8). This trend was not significant until an outlier at −2.0°h was removed ($P = 0.021$, $r^2 = 0.56$, $n = 9$). Pereiopod autotomy probably influenced these weight outcomes. The weight changes of juvenile crabs that did not molt were correlated with righting response measured immediately after exposure ($P = 0.018$, $r^2 = 0.88$, $n = 5$, $Y = a + bx^3$). Changes in weight of adult crabs were not correlated with exposure ($P \geq 0.07$, $r^2 = 0.08$, $n = 44$).

Reproduction

Exposure of ovigerous female crabs to cold air generally did not affect the eggs or subsequently released zoeae unless the female died; all eggs died if the female died. Timing of initial zoeal release (20 April ± 1 day), duration of release (11 ± 1 day), and median release date (26 April ± 1 day) did not vary with exposure ($r^2 = 0.04$, $n = 44$; Fig. 9). Zoeae placed in separate containers for two days were not significantly affected by exposure prior to hatching ($P = 0.425$, $r^2 = 0.02$, $n = 43$), and 87 ±3% continued swimming through the test period. Larval mortality, measured as the percentage of zoeae that sank to tank bottoms and died (0.4 ±0.2%), did not vary with exposure ($r^2 = 0.03$, $n = 44$). Zoeal mortality (2 ±2%) during swimming tests was not correlated with exposure ($r^2 = 0.09$).

The percentage of eggs that hatched may have been slightly affected by exposure, but our results were inconclusive ($P_{regression:arcsin}$=0.036, but $P_{lack\ of\ fit}$<0.019, and r^2=0.10). The percentage of eggs hatching in the –5.3°h treatment differed significantly from the control (Dunnett's test), but the difference was minor (99.1% versus 99.8% hatching).

Egg extrusion may have been influenced by exposure, but the data were inconsistent. Elapsed time between larval hatching and subsequent egg extrusion tended to be prolonged by exposure, but the response was variable (P_{linear}=0.005, $P_{lack\ of\ fit}$=0.719, r^2=0.19, n=41). Egg extrusion generally occurred two days (median) after zoeal release but ranged from 0 to 18 days; only crabs in the two most severe treatments (≤–4.3°h) exceeded nine days. The date of extrusion (4 May ±7 days) may also have been changed by exposure, but again the statistical results were inconclusive (P_{linear}=0.011, $P_{lack\ of\ fit}$=0.700, r^2=0.16, n=41). Exposure did not affect the percentage of Tanner crabs extruding eggs (93%, P_{linear}=0.730, r^2=0.03, n=6).

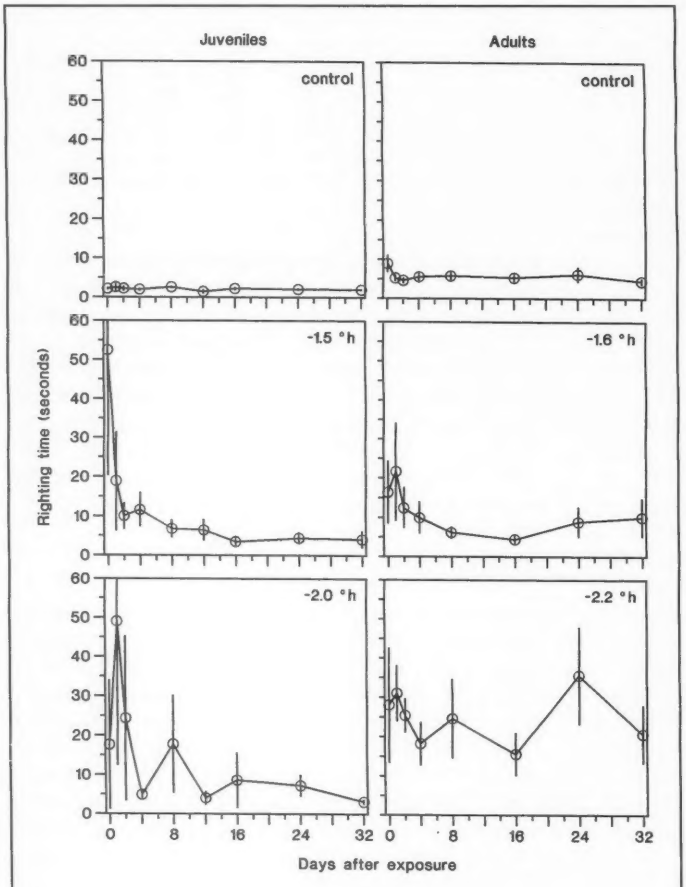

Figure 4

Righting times of juvenile and adult *Chionoecetes bairdi* as a function of time in days after emersion. Error bars are ±1 standard error.

Discussion

Extreme exposure to cold air was lethal to Tanner crabs. It is also possible that thermal shock caused when the crabs were returned to water following exposure was also damaging. Following sublethal exposure, crabs exhibited a slowed righting response, autotomy of pereiopods, depressed feeding rates (adults), and weight loss or reduced weight gain (juveniles).

Temperature and duration of treatment were both critical factors in determining how aerial exposure affected Tanner crabs. In a similar experiment with king crabs, the response of crabs to exposure was clearly observed when exposure was defined as the product of temperature and the length of exposure time (Carls and O'Clair, 1990). The use of this composite variable worked well with the current data set. However, our approach may not be generally applicable (for discussion see Carls and O'Clair, [1990]).

Design of this experiment precluded independent analysis of temperature and time factors. However, either factor may be predicted as a function of the other. For example, at –10°C, 10% of juvenile crabs may be killed by an 8-minute exposure, and 50% may be killed by a 20-minute exposure. Similarly, a 10-minute exposure would impair righting in 50% of juvenile crab at –7°C. Predicted times and temperatures were calculated from degree-hours causing death (LC) or from effective degree-hours causing cessation of righting (EC) estimates (Tables 2 and 3); temperature multiplied by time (units are Celcius and hours) matched the LC or EC estimates. Predictions of adult and juvenile Tanner crab response are summarized in Figure 10 and Appendices. In our example (Fig. 10), short-term effects are predicted by ability to right immediately after exposure; impaired crabs may be subject to increased predation at this time. Long-term effects are pre-

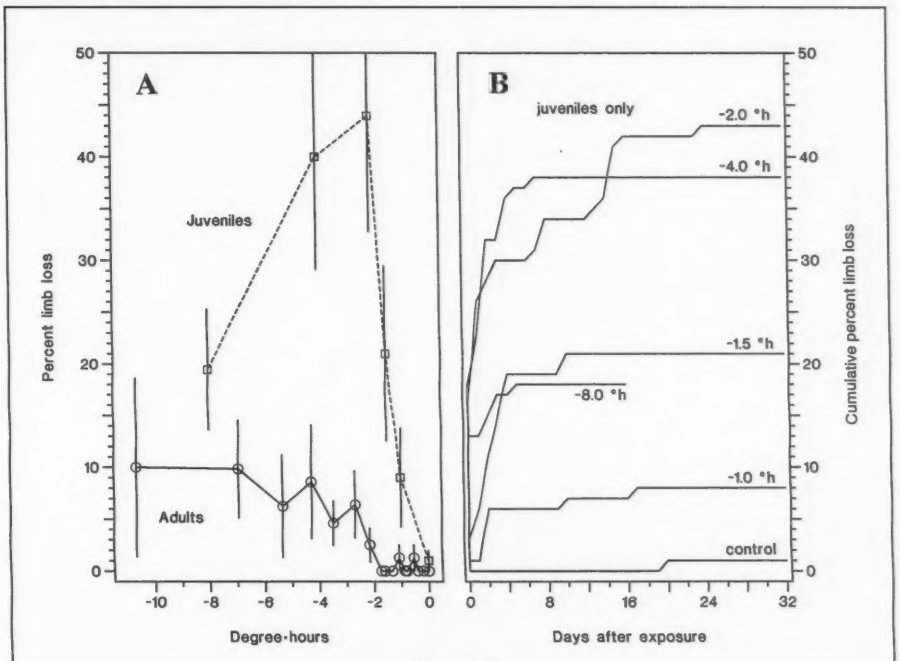

Figure 5

Percent of total pereiopod loss by juvenile and adult female Tanner crabs as a function of exposure (**A**) and as a function of time for juveniles (**B**). Error bars are ±1 standard error.

dicted by mortality after exposure-induced death ceased.

Mortality of adult Tanner crabs was significantly greater below –3°h and vigor was reduced below –2°h compared with control crabs. Exposures that are this severe probably occur infrequently on the fishing grounds except during winter in the northern Gulf of Alaska and the Bering Sea. Data are lacking on the time incidentally captured crabs remain on deck before being released, but duration probably varies widely. Larger vessels employing assembly-line techniques may process crabs more rapidly than do smaller vessels. Poor handling of culls combined with prolonged exposure may further reduce survival of incidentally caught crabs.

Crabs captured incidentally during trawling are probably stressed more than those caught in pots. Stevens (1990) reported trawl tows ranging up to 6.4 hours and retention times of Tanner crabs up to 17 hours; the median lethal holding time for Tanner crabs was 8.3 hours. Net type influenced survival, and injuries were present in a greater proportion of dead than of live crabs (Stevens, 1990).

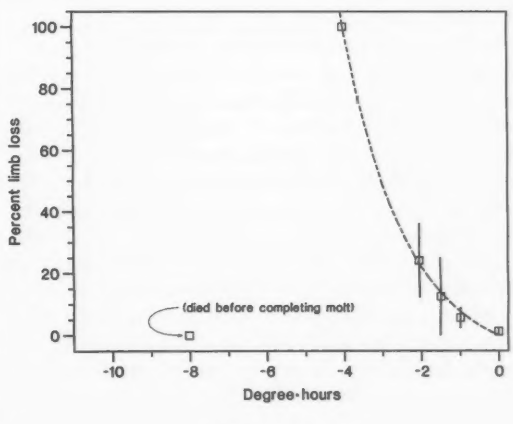

Figure 6

Limb loss by juvenile *Chionoecetes bairdi* at ecdysis as a function of exposure: percent loss=$-8.856 + 4{,}756.960\ e^{-0.629 \times (°h + 10)}$. Error bars are ±1 standard error. Numbers molting in each group were 4, 4, 2, 6, 1, and 1 for controls through –8°h, respectively.

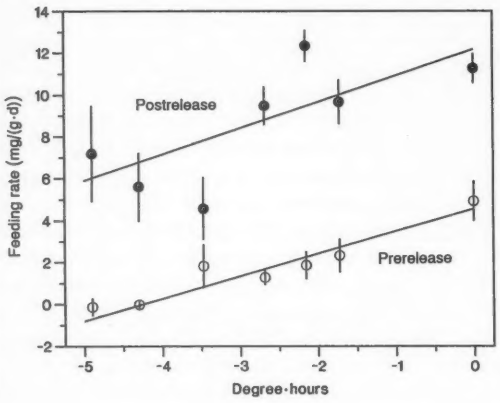

Figure 7

Feeding rates (milligram food/gram crab weight/day) of adult female *Chionoecetes bairdi* before (F_{pre}=4.556 + 1.071 × °h) and after (F_{post}=12.161 + 1.255 × °h) zoeal hatching as a functions of exposure (°h). Error bars indicate 95% CI.

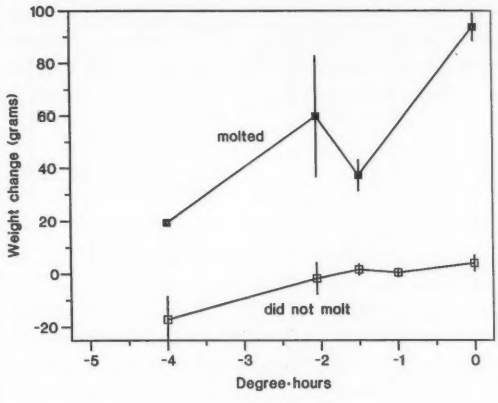

Figure 8

Changes in wet weight of juvenile *Chionoecetes bairdi* as a function of exposure. Weights of crabs that molted versus those that did not were treated separately. Error bars are ±1 standard error.

Mortality and injury due to aerial exposure have been reported for other commercially harvested decapod crustaceans. For example, king crabs were affected by exposure to cold air, but were less sensitive than Tanner crabs (Carls and O'Clair, 1990). The western rock lobster, *Panulirus cygnus*, was significantly affected by ≥15 minutes exposure to warm air (27–35°C); recapture rates were lower than for unexposed controls, and the probability of mortality due to predation rose (Brown and Caputi, 1983).

We do not know what physiological mechanism(s) caused the abnormal events during ecdysis that often resulted in death. O'Brien et al. (1986) induced apolysis (the separation of integumentary tissues from the exoskeleton during proecdysis) in several species of brachyurans by packing crabs in ice. Apolysis occurred within one hour in most cases and was not caused by death (O'Brien et al., 1986). O'Brien et al. (1986) did not observe ecdysis in their experimental crabs; therefore, the effect of apolysis on the timing, duration, and success of ecdysis in crabs is not known. In the present experiment, although mortality occurred during ecdysis in juvenile crabs, the timing of ecdysis was not affected.

Evaporative water loss during exposure probably did not contribute significantly to the effects we observed. The fact that warmer exposures, such as the 32-minute exposure at +5°C, caused little or no effect supports this supposition. Similar observations were made for king crab (Carls and O'Clair, 1990). In a study by Taylor and Whiteley (1989), the lobster *Homarus gammarus vulgaris*, which rarely comes in contact with air in its natural environment, was exposed to air at 15°C for up to 14 hours. Water loss, inferred from the constancy of most hemolymph ion concentrations, was minimal (Taylor and Whiteley, 1989). Oxygen delivered to *H. gammarus* tissues was substantially reduced, and CO_2 accumulated, but levels returned rapidly to normal after a 14-hour exposure. Lactate levels increased, but elevation of bicarbonate ions increased the buffering capacity of the hemolymph. Because exposures did not exceed 32 minutes, it is unlikely that reduced oxygen directly caused Tanner crab mortality in our experiment. However, at low air temperatures, gills may have been damaged by frost, thus impairing respiratory gas, metabolite, and ion exchange after the crabs were returned to the water.

The ability of crabs to right themselves proved to be a sensitive measure of crab viability. Righting response data collected immediately after exposure correlated strongly with less-immediate responses such as mortality and growth. Pereiopod loss also impaired righting.

Pereiopod autotomy in adult crabs was a function of exposure. Mortality may have influenced the autotomy response curve for juvenile crabs: during severe exposure, crabs apparently died before autotomy could take place.

Aerial exposure reduced weight gain in juvenile crabs and caused weight loss in juveniles that did not molt. However, wet weights of the adult crabs (all anecdysial) did not vary with exposure. This ab-

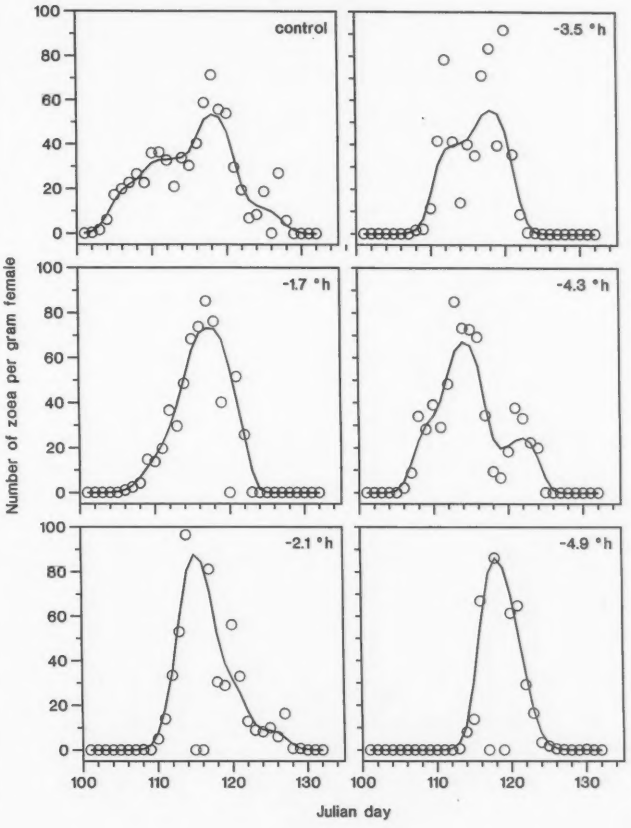

Figure 9

Zoeal production (number of zoeae per gram female *Chionoecetes bairdi*) as a function of time. Curves were fit with smoothing techniques (4253H) (Velleman and Hoaglin, 1981). Units are in °h.

sence of weight changes in the adults is puzzling because feeding rates were significantly depressed by exposure. Growth of adult western rock lobsters was reduced by exposure (Brown and Caputi, 1985).

Body size, shape, and volume may be important factors in predicting crab response to cold-air exposure. Results of the present experiment support this hypothesis: smaller crabs (juveniles) were more sensitive to exposure than were larger crabs (adults). Additionally, adult Tanner crabs were more sensitive to exposure than were larger king crabs (Carls and O'Clair, 1990), but unknown interspecific factors may have influenced this difference. An experiment involving a broad size range of conspecific individuals is needed to test whether sensitivity to exposure is size-dependent in crabs.

Surprisingly, aerial exposure did not measurably affect the developing larvae of exposed females unless the female died. Surviving crabs produced normal zoeae. Moreover, the timing of larval release, larval swimming ability, and viability were not affected by exposure. Longer-term larval responses, such as survival past the first molt and zoeal growth, were not examined. Exposure may have reduced hatching success (by <1%) of the Tanner crab larvae and possibly may have affected the timing of egg extrusion, but these responses did not vary strongly. Schlieder (1980) reported a 13% reduction in hatching success in the stone crab, *Menippe mercenaria*, compared with controls when the crabs were exposed to air at 27–33°C for two hours. Hatching success was reduced further by a five-hour exposure and by autospasy (Schlieder, 1980).

In summary, although environmental conditions as severe as those tested are uncommon on the fishing grounds during fishing operations (except in the central and northern Bering Sea), low-temperature aerial exposure during fishing operations can adversely affect incidentally captured crabs. Exposure to cold air reduced crab vigor and feeding rates, caused limb autotomy, and killed the crabs in severe situations. Progeny died if exposure to cold air killed females brooding them, otherwise larvae were not measurably affected. Prompt return of incidentally caught Tanner crabs to the sea, especially during extremely cold weather, should reduce adverse effects of exposure to cold air.

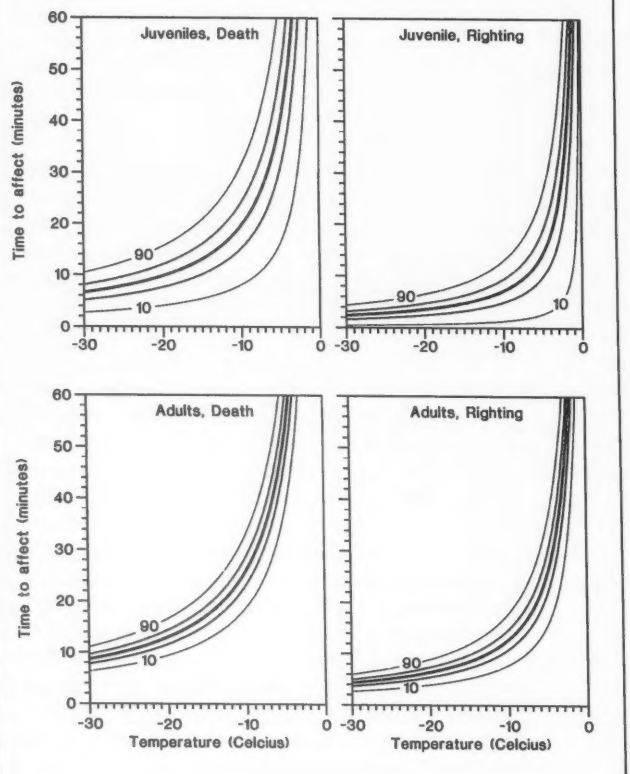

Figure 10

Predicted time in minutes required to cause death or impair righting of juvenile and ovigerous female *Chionoecetes bairdi* following exposure to cold air. Mortality predictions are based on cumulative mortality through day 16; no deaths were observed in the ensuing 16 days. Righting predictions are based on responses immediately after exposure; there was a tendancy for righting times to improve after exposure, but improvements were generally not statistically significant.

Acknowledgments

We thank Tyrus Brouillette for his technical assistance during this experiment and the reviewers who improved this manuscript with their helpful suggestions.

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Appendix Table 1

Predicted time in minutes required to cause death of the listed percentage of adult *Chionoecetes bairdi* at indicated temperatures (°C). Calculations are based lethal responses (LC10, LC30, . . . LC90) estimated on day 16.

Temperature	10%	30%	50%	70%	90%	Temperature	10%	30%	50%	70%	90%
–1.0	189	233	260	288	332	–16.0	12	15	16	18	21
–2.0	95	116	130	144	166	–17.0	11.1	13.7	15.3	16.9	19.5
–3.0	63	78	87	96	111	–18.0	10.5	12.9	14.5	16.0	18.4
–4.0	47	58	65	72	83	–19.0	9.9	12.3	13.7	15.2	17.5
–5.0	38	47	52	58	66	–20.0	9.5	11.6	13.0	14.4	16.6
–6.0	32	39	43	48	55	–21.0	9.0	11.1	12.4	13.7	15.8
–7.0	27	33	37	41	47	–22.0	8.6	10.6	11.8	13.1	15.1
–8.0	24	29	33	36	41	–23.0	8.2	10.1	11.3	12.5	14.4
–9.0	21	26	29	32	37	–24.0	7.9	9.7	10.9	12.0	13.8
–10.0	19	23	26	29	33	–25.0	7.6	9.3	10.4	11.5	13.3
–11.0	17	21	24	26	30	–26.0	7.3	9.0	10.0	11.1	12.8
–12.0	16	19	22	24	28	–27.0	7.0	8.6	9.6	10.7	12.3
–13.0	15	18	20	22	26	–28.0	6.8	8.3	9.3	10.3	11.9
–14.0	14	17	19	21	24	–29.0	6.5	8.0	9.0	9.9	11.4
–15.0	13	16	17	19	22	–30.0	6.3	7.8	8.7	9.6	11.1

Appendix Table 2

Predicted time in minutes required to cause death of the listed percentage of juvenile *Chionoecetes bairdi* at indicated temperatures (°C). Calculations are based lethal responses (LC10, LC30, . . . LC90) estimated on day 16.

Temperature	10%	30%	50%	70%	90%	Temperature	10%	30%	50%	70%	90%
–1.0	81	152	196	241	311	–16.0	5.1	9.5	12.3	15.0	19.5
–2.0	41	76	98	120	156	–17.0	4.8	8.9	11.5	14.2	18.3
–3.0	27	51	65	80	104	–18.0	4.5	8.4	10.9	13.4	17.3
–4.0	20	38	49	60	78	–19.0	4.3	8.0	10.3	12.7	16.4
–5.0	16	30	39	48	62	–20.0	4.1	7.6	9.8	12.0	15.6
–6.0	14	25	33	40	52	–21.0	3.9	7.2	9.3	11.5	14.8
–7.0	12	22	28	34	44	–22.0	3.7	6.9	8.9	10.9	14.2
–8.0	10	19	25	30	39	–23.0	3.5	6.6	8.5	10.5	13.5
–9.0	9	17	22	27	35	–24.0	3.4	6.3	8.2	10.0	13.0
–10.0	8	15	20	24	31	–25.0	3.2	6.1	7.8	9.6	12.5
–11.0	7.4	13.8	17.8	21.9	28.3	–26.0	3.1	5.8	7.5	9.3	12.0
–12.0	6.8	12.7	16.4	20.1	26.0	–27.0	3.0	5.6	7.3	8.9	11.5
–13.0	6.2	11.7	15.1	18.5	24.0	–28.0	2.9	5.4	7.0	8.6	11.1
–14.0	5.8	10.8	14.0	17.2	22.2	–29.0	2.8	5.2	6.8	8.3	10.7
–15.0	5.4	10.1	13.1	16.0	20.8	–30.0	2.7	5.1	6.5	8.0	10.4

Appendix Table 3

Predicted time in minutes required to impair righting response of the listed percentage of adult *Chionoecetes bairdi* at indicated temperatures (°C). Calculations are based on righting responses (EC10, EC30, . . . EC90) estimated immediately after exposure.

Temperature	10%	30%	50%	70%	90%	Temperature	10%	30%	50%	70%	90%
–1.0	79	109	129	148	179	–16.0	4.9	6.8	8.0	9.3	11.2
–2.0	39	55	64	74	89	–17.0	4.6	6.4	7.6	8.7	10.5
–3.0	26	36	43	49	60	–18.0	4.4	6.1	7.2	8.2	9.9
–4.0	20	27	32	37	45	–19.0	4.1	5.8	6.8	7.8	9.4
–5.0	16	22	26	30	36	–20.0	3.9	5.5	6.4	7.4	8.9
–6.0	13	18	21	25	30	–21.0	3.7	5.2	6.1	7.0	8.5
–7.0	11	16	18	21	26	–22.0	3.6	5.0	5.9	6.7	8.1
–8.0	10	14	16	19	22	–23.0	3.4	4.8	5.6	6.4	7.8
–9.0	9	12	14	16	20	–24.0	3.3	4.6	5.4	6.2	7.5
–10.0	8	11	13	15	18	–25.0	3.1	4.4	5.1	5.9	7.2
–11.0	7.1	9.9	11.7	13.5	16.3	–26.0	3.0	4.2	5.0	5.7	6.9
–12.0	6.5	9.1	10.7	12.3	14.9	–27.0	2.9	4.0	4.8	5.5	6.6
–13.0	6.0	8.4	9.9	11.4	13.8	–28.0	2.8	3.9	4.6	5.3	6.4
–14.0	5.6	7.8	9.2	10.6	12.8	–29.0	2.7	3.8	4.4	5.1	6.2
–15.0	5.2	7.3	8.6	9.9	11.9	–30.0	2.6	3.6	4.3	4.9	6.0

Appendix Table 4

Predicted time in minutes required to impair righting response of the listed percentage of juvenile *Chionoecetes bairdi* at indicated temperatures (°C). Calculations are based on righting responses (EC10, EC30, . . . EC90) estimated immediately after exposure.

Temperature	10%	30%	50%	70%	90%	Temperature	10%	30%	50%	70%	90%
–1.0	13	49	71	93	129	–16.0	0.83	3.05	4.45	5.84	8.07
–2.0	7	24	36	47	65	–17.0	0.78	2.87	4.19	5.50	7.59
–3.0	4	16	24	31	43	–18.0	0.74	2.71	3.95	5.19	7.17
–4.0	3.3	12.2	17.8	23.4	32.3	–19.0	0.70	2.57	3.75	4.92	6.79
–5.0	2.7	9.8	14.2	18.7	25.8	–20.0	0.66	2.44	3.56	4.67	6.45
–6.0	2.2	8.1	11.9	15.6	21.5	–21.0	0.63	2.33	3.39	4.45	6.15
–7.0	1.9	7.0	10.2	13.4	18.4	–22.0	0.60	2.22	3.23	4.25	5.87
–8.0	1.7	6.1	8.9	11.7	16.1	–23.0	0.58	2.12	3.09	4.06	5.61
–9.0	1.5	5.4	7.9	10.4	14.3	–24.0	0.55	2.04	2.97	3.90	5.38
–10.0	1.3	4.9	7.1	9.3	12.9	–25.0	0.53	1.95	2.85	3.74	5.16
–11.0	1.2	4.4	6.5	8.5	11.7	–26.0	0.51	1.88	2.74	3.60	4.96
–12.0	1.1	4.1	5.9	7.8	10.8	–27.0	0.49	1.81	2.64	3.46	4.78
–13.0	1.0	3.8	5.5	7.2	9.9	–28.0	0.47	1.74	2.54	3.34	4.61
–14.0	0.9	3.5	5.1	6.7	9.2	–29.0	0.46	1.68	2.45	3.22	4.45
–15.0	0.88	3.26	4.74	6.23	8.60	–30.0	0.44	1.63	2.37	3.12	4.30

Demographic analysis of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, in the Gulf of Mexico

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Abstract.—The Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, is a small coastal species caught in recreational fisheries and as bycatch in the shrimp trawl and longline fisheries in the Gulf of Mexico. Demographic analyses incorporating the best available information on validated age and growth, age at maturity (t_{mat}), maximum age (t_{max}), reproductive habits, and age-specific natural mortality and fecundity were performed. An initial set of three life history tables based on input parameters t_{mat}=4, t_{max}=10, constant age 1+ survivorship (S=0.657), and varying first year survivorship (S_0=0.432, scenario 1; S_0=0.512, scenario 2; S_0=0.657, scenario 3 or best case scenario) yielded net reproductive rates (R_0) ranging from 0.844 to 1.284, a generation length (G) of 5.8 years, and instantaneous rates of population change (r) ranging from –0.029 to 0.044. Further simulations were performed to test the sensitivity of the computed demographic parameter values to modifications in various input biological parameter values (scenarios 4 through 14). Overall, manipulations of biological parameters m_x, t_{mat}, and t_{max} caused large variations in demographic parameters r, t_{x2}, and R_0, while G remained relatively stable. All the demographic parameters proved more sensitive to changes in S than to changes in S_0. The initial set of analyses (scenarios 1 through 3) was then rerun with the estimated mean fishing mortality from 1986 to 1989 (F=0.428) added to natural mortality. Age 6+ sharks can enter the fishery under the best case scenario only to allow the population to replace itself. Ages at first capture (A_{rep}) with F=0.428 that would allow full population replacement were also calculated for scenarios 4 through 14. This study indicates that management of *R. terraenovae* under the Federal Management Plan (FMP) for sharks of the Atlantic Ocean is based on unrealistic biological characteristics for this species.

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The Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, is an abundant coastal carcharhinid species found in shelf waters of the western North Atlantic and Gulf of Mexico (Compagno, 1984), reported to reach a maximum size of approximately 110 cm total length (Compagno, 1984). Although not targeted by any U.S. commercial fisheries, in the Gulf of Mexico it is caught in recreational fisheries and discarded as bycatch in the shrimp trawl fishery (NMFS, 1993) and shark and reef fish longline fisheries (person. obs.). However, age at first entry in the various fisheries is unknown. *R. terraenovae* is grouped under the "small coastal" species category in the Federal Management Plan (FMP) for sharks of the Atlantic Ocean, which determined that this species group was not overfished, based on a stock assessment resulting in an estimate of finite rate of population increase (e^r) of 1.91. Thus, no quotas or size limits exist for this species despite its importance in several fisheries.

Biological and life history characteristics of *R. terraenovae* in the Gulf of Mexico are now well documented (Parsons, 1983, 1985; Branstetter, 1986, 1987). However, this information has not yet been applied to analyses of the population dynamics of this species, nor have the results of such analyses been published. Furthermore, as is the case with most shark species, sufficient information necessary for stock assessment is lacking (Hoff, 1990). Because long-term records of catch and effort or the age composition by species are not available, traditional surplus production models or more elaborate age-structured methods of stock assessment have seldom been used for sharks. Owing to the paucity of fisheries data, several investigators have used demographic analysis to gain insight into the population dynamics and exploitation rates of shark resources. This type of analysis has been utilized to construct life history tables or Leslie matrices (Caughley, 1977; Krebs, 1985), which are summaries of age-specific mortality and fertility rates operating on a population with the assumption of a stable age distribution. This technique allows estimation of parameters important to the dynamics of any given population. Thus, Hoenig and Gruber (1990), Cailliet (1992), and Cailliet et al. (1992) produced estimates of population dynamics by applying a demographic analysis of the lemon shark, *Negaprion brevirostris*, the leopard shark, *Triakis semifasciata*, and the angel shark, *Squatina californica*, respectively. Hoff (1990), in addition, estimated maximum sustainable yield for the sandbar shark, *Carcharhinus plumbeus*, in a modified stock production model incorporating life history information.

This study was prompted by the existence of validated age and growth data (Branstetter and McEachran, 1986) and reproductive information (Parsons, 1983) on *R. terraenovae*, which provided several important parameters needed for construction of a life history table, i.e. lifespan, fecundity, and age at maturity.

The purpose of this study was 1) to produce, using the life history table approach and the best biological information available, reliable estimates of demographic parameters for *R. terraenovae* in the Gulf of Mexico, 2) to assess the sensitivity of the computed demographic parameters of the population to a variety of biological (input) parameter manipulations and harvest scenarios, and 3) to compare the resultant rates of population increase with that calculated for the "small coastal" shark group in the FMP for sharks of the Atlantic Ocean and to evaluate the biological basis of the stock assessment on which present management measures are based.

Materials and methods

Life history tables incorporating the best biological information available on *R. terraenovae* in the Gulf of Mexico were constructed. Maximum age (lifespan or longevity; t_{max}) has been estimated to be 8 to 10 years (Branstetter, 1987), and age at maturity (t_{mat}) for females has been estimated at 4 years (Branstetter, 1987), and from 2.4 to 3.9 years (Parsons, 1985). For this study, it was assumed that all females reproduced after reaching maturity. Parsons (1983) reported that parturition was annual, gestation period was 10 to 11 months, and sex ratios at birth were 1:1. He also found a significant relationship between total length of gravid females and number of offspring produced. Fecundity at size was calculated from the regression equation $Y = -8.4109 + 0.1396X$ (r=0.50, P<0.001, n=78; Parsons[1]), where X is female total length and Y is number of offspring. Female length at age was obtained from the von Bertalanffy growth function for both sexes combined derived by Branstetter (1987): $L_t = L_\infty (1-e^{-K(t-t_0)})$, where K=0.359, L_∞=108, and t_0=–0.985. Number of offspring was further divided by two, because the natality function (m_x) at age represents the number of female offspring per female parent and sex ratios at birth are 1:1 and parturition is annual (Parsons, 1983). Reports of unusually large litter sizes in tropical populations of *R. terraenovae* were also incorporated in some of the analyses that follow by doubling fecundity at age (m_x).

[1] Parsons, G. Univ. Mississippi, MI 38677. Personal commun., 1993.

The instantaneous natural mortality rate (M) was calculated from Hoenig's (1983) equation relating maximum age to total mortality rate, derived from data pertaining to unexploited or lightly exploited stocks. A value of 0.42 for Z (instantaneous total mortality rate) was derived from the regression equation $\ln(Z) = 1.46 - 1.01 \ln(t_{max})$, where t_{max} is longevity in years. Assuming that maximum age was determined from a time when there was no fishing directed at this species, Z can be approximated to M. The proportion of survivors at the start of each age interval (x) was $l_x = N_0(e^{-Mx})$, where N_0 is the number of individuals at time 0.

Demographic parameters were computed following methodology by Krebs (1985) and included R_0 (net reproductive rate per generation), G (generation length in years), and r (intrinsic rate of population change). All the values of r reported in this study were refined by using the Euler equation (Wilson and Bossert, 1971; Krebs, 1985):

$$\sum_{x=0}^{\infty} l_x m_x e^{-rx} = 1.$$

The finite or annual rate of change (e^r) was then calculated from the refined values of r. In addition, the theoretical population doubling or halving time in years ($t_{\times 2}$) assuming a stable age distribution was computed as $(\ln 2)/r$ or $(\ln 0.5)/r$, respectively (Krebs, 1985).

The initial set of analyses, consisting of three different scenarios, was run by using the most reliable input biological parameters: t_{max}=10, t_{mat}=4, m_x=baseline age-specific natality, and S=0.657. In scenario 1, first year natural mortality was arbitrarily doubled (M=0.42 × 2=0.84) or S_0=0.432. In scenario 2, a value of S_0=0.512 was obtained from the Leslie matrix algorithm by assuming an equilibrium (or stationary) population (Vaughan and Saila, 1976). Thus, the following equation was solved for S_0 after assuming r=0:

$$S_o = \frac{e^r}{m_1 + \sum_{i=1}^{I-1}\left[\left(m_{i+1}e^{-ir}\right)\left(\prod_{j=1}^{i} S_j\right)\right]},$$

where m_i is fecundity at age, I is the oldest age group in the population (10 years), and S_j is survival from age j to j+1. In scenario 3, (referred to as the best case scenario), S_0 was assumed to be equal to survivorship in the following years (S_0=S=0.657=$e^{-0.42}$). For this best case scenario, the stable age distribution (C_x) was calculated according to Krebs (1985) and plotted.

In a second set of analyses, the input biological or life history parameters (t_{mat}, t_{max}, m_x, S, S_0) were varied to test the sensitivity of the resultant demographic parameters (R_0, G, r, and $t_{\times 2}$). These sensitivity analyses measured the percentage change of the output de-

mographic parameter of interest relative to the best case scenario. In the case of $t_{\times 2}$, sensitivity was assessed by calculating a multiplication factor that measures the number of times the population doubling time changes relative to the best case scenario (example: if $t_{\times 2}$=15.7 in the best case scenario and 4.1 in the altered state, then the multiplication factor [mf]=15.7/4.1=3.8, i.e. $t_{\times 2}$ has been shortened 3.8 times in the altered state).

Based on the results from the initial set of analyses (scenarios 1 through 3), the existing knowledge of life history traits in *R. terraenovae*, and the results from the FMP (e^r=1.91), input parameter values were manipulated in the direction that would be favorable to population increase and should thus be regarded as optimistic scenarios. The following variations, relative to the best case scenario, were applied: doubling m_x (m_x=2; scenario 4); reducing t_{mat} by 1 year (t_{mat}=3; scenario 5) and by 2 years (t_{mat}=2; scenario 6); reducing t_{mat} by 1 year and doubling m_x (t_{mat}=3, m_x=2; scenario 7); reducing t_{mat} by 2 years and doubling m_x (t_{mat}=2, m_x=2; scenario 8); increasing S_0 by 10% (S_0=0.723; scenario 9) and by up to 50% (S_0=0.985; scenario 10); increasing S by 10% (S=0.723; scenario 11) and by up to 50% (S=0.985; scenario 12); doubling t_{max} (t_{max}=20 [note that S and S_0 will also vary, since they depend on t_{max}]; scenario 13); and an extreme manipulation that was undertaken to approximate the FMP value of e^r=1.91 (equivalent to an r of 0.647), where t_{mat} was reduced by 1 year, m_x was doubled, and S and S_0 set at 95% (t_{mat}=3, m_x=2, S=S_0=0.95; scenario 14).

A third set of simulations was run incorporating the estimated mean instantaneous fishing mortality rate from 1986 to 1989 (F=0.428), as used in the stock assessment of small coastal species (Parrack[2]) on which the FMP for sharks of the Atlantic Ocean is based, to demonstrate the effect of exploitation and various age-at-first-entry scenarios. Fishing mortality (F) was added to natural mortality (M) in the survivorship function $l_x = N_0(e^{-[M+F]x})$, with F initially starting at age 0, then sequentially up to age 9. A_{rep}, the minimum age at which individuals can first enter the fishery and still allow the population to replace itself ($r \geq 0$) was calculated by noting the age at which the intrinsic rate of increase (r) becomes zero or positive. These simulations were run first under scenarios 1 through 3, and then under scenarios 4 through 14.

Results

The initial set of life history tables yielded net reproductive rates per generation (R_0), ranging from 0.844 to 1.284, a generation length (G) of 5.8 years, and intrinsic rates of population change (r), ranging from –0.029 to 0.044 (Table 1) depending on the value of first year survivorship (S_0) used. In scenario 1 (S_0=0.432), the results indicated that the population would decrease at a rate of 2.9% per year and would halve about every 24 years. Halving times are indicated by negative values in the $t_{\times 2}$ column. In scenario 2 (S_0=0.512), r is equal to 0 by definition. Under the best case scenario (scenario 3; S_0=0.657), the

[2] Parrack, M. L. 1990. A preliminary study of shark exploitation during 1986–1989 in the U.S. FCZ. Contrib. MIA-90-493, NOAA, NMFS, SEFC, Miami, FL 33149, 23 p.

Table 1

Simulations of the Gulf of Mexico population of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, under three scenarios that use input parameter values representing the best biological information available. Only natural mortality is included in these analyses. First year survival rates (S_0) were obtained as follows: S_0=0.432 (scenario 1) was obtained by doubling the natural mortality value computed from Hoenig's (1983) relationship between mortality rate and maximum age; S_0=0.512 (scenario 2) was computed from the Leslie matrix algorithm (see text) assuming an equilibrium population (Vaughan and Saila, 1976). The third line (in italics) represents the best case scenario (scenario 3; S_0=S=0.657).

	Input parameter values[1]					Computed parameter values[2]				
Scenario	t_{mat}	t_{max}	m_x	S	S_0	R_0	G	r	e^r	$t_{\times 2}$
1	4	10	1[3]	0.657	0.432	0.844	5.762	–0.029	0.971	–23.9
2	4	10	1	0.657	0.512	1.000	5.762	0.	1.000	—
3	*4*	*10*	*1*	*0.657*	*0.657*	*1.284*	*5.762*	*0.044*	*1.045*	*15.7*

[1] t_{mat}=age at maturity; t_{max}=maximum age; m_x=age-specific natality; S=survivorship after the first year of life; S_0=survivorship for the first year of life.

[2] R_0=net reproductive rate per generation; G=generation length, in years; r=intrinsic rate of population change refined through the Euler equation (see text); e^r=finite rate of population change; $t_{\times 2}$=theoretical doubling (positive values) or halving (negative values) time in years assuming a stable age distribution.

[3] "1" indicates baseline age-specific natality.

population increased at 4.5% per year and doubled about every 16 years.

The predicted stable age distribution (C_x) for the best case scenario (Fig. 1) suggested that about 80% of the population was composed of immature individuals. Because of the lack of data on sizes and ages at first capture in the recreational and commercial fisheries, the actual proportion of the population subject to fishing is unknown. Likewise, no size or age composition of this population is available from surveys, precluding any comparisons with the theoretical C_x.

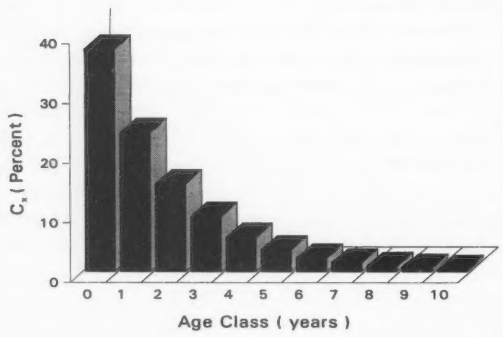

Figure 1

Predicted stable age distribution of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, under the best case scenario presented in Table 1, assuming geometric growth (with r=0.044) and constant age-specific mortality and fertility rates.

Results of the sensitivity analyses indicated that doubling age-specific natality, m_x, had a distinct effect (a 286% increase) on the population's rate of increase, r (scenario 4), and would allow the population to double in only 4.1 years or 3.8 times faster than in the best case scenario (Table 2). Generation length, G, remained the same, while net reproductive rate per generation, R_0, increased 100% (Table 3).

Decreasing age at maturity, t_{mat}, by one year (scenario 5) produced a smaller change in r, $t_{\times 2}$, and R_0 (Tables 2 and 3) than doubling m_x, but decreased G by 12% (Table 3). Further decreasing t_{mat} by another year (scenario 6) produced almost the same values of r and $t_{\times 2}$ as those obtained in scenario 4 (Table 2), although R_0 increased only 5% and G decreased by 20%. The combined effect of decreasing t_{mat} and doubling m_x together (scenarios 7 and 8) produced increases in r up to near 700% and $t_{\times 2}$ values up to 8 times shorter than in the best case scenario (Table 2). Under scenarios 7 and 8, R_0 also increased by up to more than 200%, while G decreased by up to 20%.

Increasing first year survivorship, S_0, by 10% (scenario 9) yielded a value of r 39% higher and a value of $t_{\times 2}$ 1.4 times shorter than in the best case scenario (Table 2), affected R_0 very little (a 10% increase only), and had no effect on G (Table 3). A further increase

Table 2

Simulations of the Gulf of Mexico population of *Rhizoprionodon terraenovae* to test the sensitivity of computed population rate of increase and doubling time to input biological parameter values. Input values were manipulated in scenarios 4 through 14; the best case scenario (BC; top row) is shown in italics to facilitate comparison. All other symbols are as defined in Table 1.

	Input parameter values					Computed parameter values			
Scenario	t_{mat}	t_{max}	m_x	S	S_0	r	% change of r[1]	$t_{\times 2}$	mf[2]
BC	*4*	*10*	*1*	*0.657*	*0.657*	*0.044*	—	*15.7*	—
4	4	10	2[3]	0.657	0.657	0.170	286	4.1	3.8
5	3	10	1	0.657	0.657	0.111	152	6.2	2.5
6	2	10	1	0.657	0.657	0.168	282	4.1	3.8
7	3	10	2	0.657	0.657	0.265	502	2.6	6.0
8	2	10	2	0.657	0.657	0.356	709	1.9	8.3
9	4	10	1	0.657	0.723	0.061	39	11.4	1.4
10	4	10	1	0.657	0.985	0.117	166	5.9	2.7
11	4	10	1	0.723	0.657	0.123	179	5.6	2.8
12	4	10	1	0.985	0.657	0.378	759	1.8	8.7
13	4	20	1	0.811	0.658	0.228	418	3.0	5.2
14	3	10	2	0.950	0.950	0.634	1,341	1.1	14.3

[1] % change of r relative to the best case scenario.

[2] Multiplication factor indicating the number of times $t_{\times 2}$ has been shortened relative to the best case scenario.

[3] "2" indicates baseline age-specific natality values have been doubled.

Table 3

Simulations of the Gulf of Mexico population of *Rhizoprionodon terraenovae* to test the sensitivity of computed net reproductive rate per generation and generation length to input biological parameter values. Input values were manipulated in scenarios 4 through 14; the best case scenario (BC; top row) is shown in italics to facilitate comparison. All other symbols are as defined in Table 1.

	Input parameter values					Computed parameter values			
Scenario	t_{mat}	t_{max}	m_x	S	S_0	R_0	% change of R_0[1]	G	% change of G[1]
BC	*4*	*10*	*1*	*0.657*	*0.657*	*1.28*	—	*5.76*	—
4	4	10	2[2]	0.657	0.657	2.57	100	5.76	0
5	3	10	1	0.657	0.657	1.72	34	5.06	–12
6	2	10	1	0.657	0.657	1.34	5	4.58	–20
7	3	10	2	0.657	0.657	3.43	168	5.06	–12
8	2	10	2	0.657	0.657	4.08	219	4.58	–20
9	4	10	1	0.657	0.723	1.41	10	5.76	0
10	4	10	1	0.657	0.985	1.92	50	5.76	0
11	4	10	1	0.723	0.657	2.05	60	6.06	5
12	4	10	1	0.985	0.657	11.66	809	7.20	25
13	4	20	1	0.811	0.658	4.99	290	8.30	44
14	3	10	2	0.950	0.950	29.6	2,212	6.7	16.3

[1] % change of R_0 and G relative to the best case scenario.
[2] "2" indicates baseline age-specific natality values have been doubled.

in S_0 up to 50% (scenario 10) had a more distinct effect on r (166% increase), $t_{\times 2}$ (2.7 times shorter), and R_0 (50% increase), but did not affect G (Tables 2 and 3). Increasing age 1+ survivorship (S) by 10% (scenario 11) had a similar effect on all the demographic parameters to increasing S_0 by 50% (scenario 10; Tables 2 and 3), whereas increasing S by 50% (scenario 12) had a very profound effect on all the demographic parameters, increasing r by 759%, shortening $t_{\times 2}$ by almost 9 times (similar to scenario 8), increasing R_0 by over 800% and lengthening G by 25% (Tables 2 and 3).

Doubling longevity (t_{max}) to 20 years (scenario 13) also markedly affected r (418% increase), $t_{\times 2}$ (5 times shorter), and R_0 (290% increase), and produced the largest value of G (8.3 or a 44% increase) in all scenarios (Tables 2 and 3).

Finally, the extreme manipulations of scenario 14 (reducing t_{mat} to 3 years, doubling m_x, increasing S and S_0 to 95%, with a t_{max} of 10 years) produced a 13-fold increase in r, a value of $t_{\times 2}$ more than 14 times shorter, a 22-fold increase in R_0 and only a 16.3% increase in G (Tables 2 and 3).

For all simulations, population doubling time ($t_{\times 2}$) was lessened and generation length (G) was the demographic parameter less sensitive to changes in input biological parameter values.

With the estimated mean fishing mortality from 1986 to 1989 (F=0.428) added to natural mortality starting at each age interval from 9 to 0, R_0 and r were progressively reduced as F was progressively started closer to age-0 (Table 4). In scenarios 1 (S_0=0.432) and 2 (S_0=0.512), r was always negative and became increasingly so as simulated fishing started earlier in the life of *R. terraenovae*. Only by using best case scenario (scenario 3) values could the population be made to replace itself or grow by manipulating age at first capture. When fishing pressure was applied between 6 and 5 years of age or about 97 cm total length (TL) the population was able to replace itself. Generation length remained the same under the three scenarios but progressively decreased as fishing mortality included progressively earlier ages. Theoretical halving time also progressively shortened as fishing started at younger ages, whereas in the best case scenario doubling time increased as age at first capture dropped from 9 to 6 years.

The effect of added fishing mortality on survivorship can be identified as a progressive decrease in percentage survival as fishing starts progressively earlier in the lifespan of the shark (Fig. 2). Age-specific reproduction also decreases significantly as fishing mortality is applied at progressively earlier ages (Fig. 3).

When the estimated mean fishing mortality from 1986 to 1989 (F=0.428) was added to natural mortality in scenarios 4 through 14 (Table 5), A_{rep}, the earliest age at which sharks can first be captured to

Figure 2

Survivorship curves for *Rhizoprionodon terraenovae* under the survival conditions presented in Table 1 for the best case scenario (S=S_o=0.657) and fishing mortality as in Table 4 (F=0.428), starting at three different ages (1, 5, and 8 years).

Figure 3

Age-specific reproduction for *Rhizoprionodon terraenovae* under the survival conditions presented in Table 1 for the best case scenario (S=S_o=0.657) and fishing mortality as in Table 4 (F=0.428), starting at three different ages (1, 5, and 8 years).

Table 4

Simulations of the Gulf of Mexico population of *Rhizoprionodon terraenovae* under the three same scenarios as in Table 1 but with estimated mean fishing mortality from 1986 to 1989 (F=0.428 [Parrack[2]]) added to natural mortality starting at different ages. All symbols are as defined in Table 1. Computations based on the following first year survival rates: scenario 1 (S_o=0.432); scenario 2 (S_o=0.512); and scenario 3 (best case, S_o=0.657).

Demographic parameter	Age at first capture									
	9	8	7	6	5	4	3	2	1	0
Scenario 1										
R_o	0.83	0.81	0.77	0.71	0.62	0.49	0.32	0.21	0.14	0.09
G	5.70	5.60	5.45	5.27	5.06	4.86	4.86	4.86	4.86	4.86
r	–0.03	–0.04	–0.05	–0.07	–0.09	–0.14	–0.23	–0.31	–0.38	–0.46
e^r	0.97	0.96	0.95	0.94	0.91	0.87	0.80	0.74	0.68	0.63
t_{x2}	–21.7	–18.2	–14.4	–10.7	–7.4	–4.9	–3.1	–2.3	–1.8	–1.5
Scenario 2										
R_o	0.99	0.96	0.91	0.84	0.73	0.58	0.38	0.25	0.16	0.10
G	5.71	5.60	5.45	5.27	5.06	4.86	4.86	4.86	4.86	4.86
r	–0.00	–0.01	–0.02	–0.03	–0.06	–0.11	–0.19	–0.27	–0.35	–0.43
e^r	1.00	0.99	0.98	0.97	0.94	0.90	0.82	0.76	0.70	0.65
t_{x2}	–346.6	–99.0	–40.8	–21.0	–11.4	–6.3	–3.6	–2.5	–2.0	–1.6
Scenario 3 (best case)										
R_o	1.27	1.23	1.17	1.08	0.94	0.75	0.49	0.32	0.21	0.13
G	5.71	5.60	5.45	5.27	5.06	4.86	4.86	4.86	4.86	4.86
r	0.04	0.04	0.03	0.01	–0.01	–0.06	–0.14	–0.22	–0.31	–0.38
e^r	1.04	1.04	1.03	1.01	0.99	0.94	0.86	0.80	0.73	0.68
t_{x2}	16.5	18.7	23.9	49.5	–57.8	–11.7	–4.8	–3.0	–2.2	–1.8
size[1]	105	103.7	101.9	99.2	95.4	90	82.2	71.0	55.0	32.3

[1] Total length, in cm, obtained from the von Bertalanffy growth equation relating age to length (see text).

Table 5

Simulations of the Gulf of Mexico population of *Rhizoprionodon terraenovae* under several scenarios (4 through 14) using different input biological parameter values and with fishing mortality (F=0.428) as in Table 4. The best case scenario (BC; top row) is included in italics to facilitate comparison. All other symbols are as defined in Table 1; A_{rep} is the age at which sharks can first enter the fishery and still allow the population to replace itself.

	Input parameter values					Computed parameter values					
Scenario	t_{mat}	t_{max}	m_x	S	S_0	A_{rep}	R_0	G	r	e^r	$t_{\times 2}$
BC	*4*	*10*	*1*	*0.657*	*0.657*	*6*[1]	*1.08*	*5.27*	*0.010*	*1.010*	*49.5*
4	4	10	2[2]	0.657	0.657	4	1.50	4.87	0.084	1.088	8.2
5	3	10	1	0.657	0.657	4	1.18	4.18	0.040	1.041	17.3
6	2	10	1	0.657	0.657	3	1.25	3.47	0.064	1.066	10.8
7	3	10	2	0.657	0.657	2	1.20	3.99	0.046	1.047	15.1
8	2	10	2	0.657	0.657	1	1.20	3.29	0.057	1.059	12.2
9	4	10	1	0.657	0.723	5	1.04	5.06	0.007	1.007	99.0
10	4	10	1	0.657	0.985	4	1.12	4.86	0.024	1.024	28.9
11	4	10	1	0.723	0.657	4	1.09	5.01	0.017	1.017	40.8
12	4	10	1	0.985	0.657	1	1.15	5.70	0.025	1.025	27.7
13	4	20	1	0.811	0.658	3	1.16	5.33	0.028	1.028	24.7
14	3	10	2	0.950	0.950	0	2.56	4.86	0.206	1.229	3.4

[1] Under this scenario, sharks can enter the fishery at age 6 and above with a fishing mortality rate of F=0.428 (F=0 for all sharks ages 5 and below) added to the natural mortality rate and still allow the population to replace itself ($r \geq 0$).

[2] "2" indicates baseline age-specific natality values have been doubled.

allow for full population replacement ($r \geq 0$) given the fishing mortality, became progressively smaller as the value of r increased (see Table 2 for reference). Increasing S_0 by 10% (scenario 9) allowed for an age at first capture of 5 years, while doubling m_x (scenario 4), reducing t_{mat} to 3 years of age (scenario 5), increasing S_0 by 50% (scenario 10), or increasing S by 10% (scenario 11) all had the same effect of allowing for an age at first capture of 4 years (90 cm TL) compared with 6 years (99 cm TL) under the best case scenario. Reducing t_{mat} by 2 years (scenario 6) or increasing t_{max} to 20 years (scenario 13) both allowed for an A_{rep} of 3 years (82 cm TL), while reducing t_{mat} by 1 year and doubling m_x (scenario 7) allowed an A_{rep} of 2 years. Under the most extreme manipulations, which included reducing t_{mat} by 2 years and doubling m_x (scenario 8), increasing S by 50% (scenario 12), and reducing t_{mat} to 3 years, doubling m_x, and setting S and S_0 at 95% (scenario 14), an age at first capture of 1 year (55 cm TL; scenarios 8 and 12) and of 0 years (32 cm TL) could be applied in a given year.

Discussion

These demographic analyses using the best available information indicate that the Gulf of Mexico population of *R. terraenovae* may be very vulnerable to fishing pressure. Results showed that, based on known life history parameters, the population's intrinsic rate of increase was, at best, only r=0.044, equating to a finite rate of e^r=1.045, which is much lower than the rate estimated for "small coastal" species in the stock assessment used to develop the FMP for sharks of the Atlantic Ocean (e^r=1.91). Furthermore, comparable rates to the FMP values were only obtained after extreme manipulations of the input life history parameters, which diverged too widely from observed life history parameters to be realistic. For example, one of the possible scenarios that would yield an estimate of e^r of 1.91 implies that age at maturity has to be decreased from 4 to 3 years, fertility doubled, and survivorship increased by almost 50% relative to the most optimistic initial scenario, i.e. the best case scenario, resulting in estimates of 29.6 for R_0, 6.7 for G, and 1.1 for $t_{\times 2}$. This means that, in the absence of fishing, the population would almost double every year.

Rhizoprionodon terraenovae is the main species caught in the Texas recreational shark fishery and is also caught by the headboat and other recreational fisheries in the Gulf of Mexico. More importantly, it represents a significant bycatch in the shrimp trawl fishery operating in the Gulf of Mexico and to a lesser extent in the longline reef fish and shark fisheries, and in the gillnet fishery in the same area. The lack of data on the age and size at which individuals of *R.*

terraenovae first enter these fisheries, as well as the relative proportions of each age and size group represented, preclude a more detailed analysis at this time. However, the demographic analysis representing the best case scenario indicated that under the present fishing level *R. terraenovae* should not enter the fishery until individuals reach about 97 cm TL or almost 6 years of age if the population was managed to just replace itself. There is evidence that smaller animals are being caught in the various fisheries, but the proportions of each age class are unknown.

The biological parameters incorporated in scenarios 1 through 3 represent the best, most reliable information available. Data on age and growth were taken from a tetracycline-validated laboratory study (Branstetter, 1987) which indicated that females mature entering their fifth year of life (age-4) and that maximum age is between 8 and 10 years. In another study (Parsons, 1985), female maturity was estimated at between 2.4 to 3.9 years. However, this study used only males and mean lengths for age classes, which, as pointed out by Branstetter (1987), affected the von Bertalanffy parameters. The possibility of earlier female age at maturity and even longer lifespan was incorporated in several of the demographic analyses (scenarios 5, 6, 7, 8, 13, and 14), which evidently yielded more liberal results on which more risk-prone management decisions could be based.

The unpublished information on fertility at age was derived from a study on the reproductive biology of *R. terraenovae* (Parsons, 1983) and relates female total length to number of uterine eggs or embryos for 78 specimens. Parsons (1983) also noted that tropical populations of *R. terraenovae* had been reported to have as many as 12 embryos. This possibility was taken into account by doubling fertility at age in several analyses (scenarios 4, 7, 8, and 14), which again produced more optimistic estimates of population parameters.

The age, growth, and reproduction information used in this study was based on animals collected in the northern central and western Gulf of Mexico. The extent to which this information is applicable to the entire population or whether there are different stocks in the Gulf with different age, growth, and reproductive capabilities is not known. For example, I recently examined an 82-cm-TL pregnant female with 3 embryos, measurements which fit nicely the regression equation of Parsons (1983), but which would result in a back-calculated age of 3 years with the von Bertalanffy growth function, although the female could have been older, e.g. age 4, owing to variability in size at age, which is not uncommon in sharks (Kusher et al., 1992, and references therein).

The most important and also the most difficult parameter to estimate is natural mortality (M). The value of M used in this study was taken from Hoenig's (1983) relationship between longevity and total mortality for virgin or lightly exploited stocks. The assumption that Z could be approximated to M, or that no fishing mortality occurred during the period for which growth parameters for this species were derived, may have been violated. However, the possibility of lower natural mortality values was incorporated in several analyses (scenarios 9 through 14). While Hoenig's equation represents a shortcut and obvious simplification of reality, the lack of catch and effort data, or age or size composition for stocks of this species precludes calculation of any other estimates of M at this time. Lack or inappropriateness of both fishery and biological data may explain why several other researchers have used the same approach to estimate natural mortality in shark population studies. Except for age-0 *Negaprion brevirostris* (Manire and Gruber, 1993), no actual age-specific estimates of natural mortality are available for any shark species.

The value derived for M (0.42) in this study is equivalent to an annual survivorship of 0.66, which is low when compared with survival estimates for other species of sharks. Values derived from Hoenig's (1983) regression equation include 0.82 for the angel shark, *Squatina californica*, (Cailliet et al., 1992); 0.85 for *N. brevirostris* (Hoenig and Gruber, 1990); 0.87 for *Triakis semifasciata* (Smith and Abramson, 1990; Cailliet, 1992); and 0.90 for *Carcharhinus plumbeus* (Hoff, 1990). Grant et al. (1979) derived a value of 0.90 for the Australian school shark, *Galeorhinus australis*, using cohort analysis, and Walker (1992) used a value of 0.82 in a dynamic pool fishery simulation model of the gummy shark, *Mustelus antarcticus*, which was also obtained through cohort analysis. The lower survivorship value for *R. terraenovae* may be due to the smaller size of this species which would make it more susceptible to predation by other sharks, especially at early ages, since pups are born at about only 30 cm TL in coastal waters about 10 m deep (Castro, 1993).

The very high estimate of F (0.428) used in this study was derived from a shark stock assessment that is the basis for the recently implemented (26 April 1993) FMP for sharks of the Atlantic Ocean. However, the accuracy of this estimate, based on a 4-year catch-and-effort time series, is uncertain, and the demographic analyses undertaken in this study indicate that *R. terraenovae* is vulnerable to high removal levels in the early years of life.

It is also possible that the age, growth, and reproductive data used in this study are only representative of the population at a time when fishing pressure was not as high as it is at present. Potential

Table 6

Life history parameters for several species of sharks compared to the best case scenario for *Rhizoprionodon terraenovae* in the Gulf of Mexico.

Species	R_o	G	r	e^r	t_{x2}	Study
Triakis semifasciata	4.47	22.35	0.067	1.069	10.3	Cailliet, 1992
Negaprion brevirostris	1.27	16.23	0.015	1.015	46.7	Hoenig and Gruber, 1990
Squalus acanthias	3.05	49.63	0.023	1.023	30.1	Jones and Geen, 1977[1]
Carcharhinus plumbeus	1.48	21.5	0.018	1.018	38.5	Hoff, 1990
Squatina californica	2.25	14.5	0.056	1.057	12.4	Cailliet et al., 1992
Rhizoprionodon terraenovae	1.28	5.78	0.044	1.045	15.7	This study

[1] Modified by Eguchi and Cailliet (Gregor Cailliet, Moss Landing Marine Laboratories, Moss Landing, CA 95039-0450. Personal commun., 1994).

compensatory mechanisms countering stock depletion may include increased growth with corresponding earlier age at maturity, earlier size at maturity, higher fecundity, immigration of stocks from other areas, and increased survival rates through density-dependent regulation (Walker, 1992).

The estimates of demographic parameters derived under the best case scenario in this study are within the range of values derived for other species of sharks (Table 6). The estimate of R_o (1.28) for *R. terraenovae* is almost identical to that derived by Hoenig and Gruber (1990) for *N. brevirostris* (1.27), probably owing to the similar fecundity in the two species. Generation length (G) is considerably shorter in *R. terraenovae* than in any other species because of its shorter lifespan. The estimates of r and e^r fall between those for other species. These relatively low values in a fast-growing, short-lived species such as *R. terraenovae* can be explained mainly by low fecundity and high mortality levels as the demographic analyses showed. Theoretical doubling time (t_{x2}) also lies between estimates for other species of sharks.

In the light of the incomplete yet reasonable biological information available, it seems sound to conclude that *R. terraenovae*, as perhaps some of the other small coastal species included in the FMP, is a species vulnerable to fishing. This becomes especially relevant with the recent implementation of the shark FMP which has set quotas and trip limits and has provided for fisheries closures for larger coastal species while allowing the unrestricted capture of small coastal sharks, of which *R. terraenovae* is the most representative species. Increased fishing pressure on *R. terraenovae*, especially on younger animals, would certainly deplete the stocks of this species.

The present study raises some serious concerns about the use of surplus production models utilizing very short series of catch and effort data and not taking into account the biological characteristics of a species. In the particular case of *R. terraenovae*, it can lead to policies that in turn lead to overfishing and stock depletion. It is advised that bycatch of *R. terraenovae* in the shrimp fishery and other fisheries in the Gulf of Mexico, as well as in the eventual development of a directed fishery for this species, be closely monitored in the near future. Development of stock identification techniques are also needed to determine stock structure and the degree of mixing and immigration of this species into fishing areas. Updated, more complete fisheries statistics combined with improved biological information should be used to re-assess the status of *R. terraenovae* in the small coastal species group of the shark FMP for future sound management actions.

Acknowledgments

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Evaluation of a video camera technique for indexing abundances of juvenile pink snapper, *Pristipomoides filamentosus*, and other Hawaiian insular shelf fishes

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Abstract.—Bottom longline and baited video camera operations were conducted at 39 stations off the Hawaiian Islands of Oahu, Maui, and Kauai during 1992. Objectives of the 1992 cruise were to assess the precision, accuracy, and efficiency of a video camera system versus a traditional abundance index (longline catch per unit of effort [CPUE]) for juvenile pink snapper ("opakapaka"), *Pristipomoides filamentosus*, a commercially important eteline snapper in Hawaii. Precision of the video samples was reevaluated with data from 18 stations sampled during 1993 off Kaneohe Bay. The video index of the maximum number of opakapaka observed (MAXNO, the natural log-transformed mean of three camera drops) was best correlated with the log of longline CPUE (r=0.79, P<0.001, n=15). Variation in the data for video MAXNO was nominally less than that of the longline CPUE. No monotone trend over stations was noted in samples from 1993. Sample sizes of 33 stations for longline and 18 stations for video would be necessary to detect twofold changes in abundance of opakapaka at a site, based on our analysis of the two end positions of the 10 windward Oahu stations that had three quantitative deployments. Reanalysis of power with data from the 1993 cruise indicates that a sample size of approximately 22 stations (α_2=0.05) or approximately 17 stations (α_2=0.1) would be necessary to detect twofold changes.

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Deep-water snappers (family: Lutjanidae) support an important commercial fishery around the main Hawaiian Islands (MHI) and the Northwestern Hawaiian Islands (NWHI). Pink snapper (or "opakapaka"), *Pristipomoides filamentosus*, has been one of the most important species in terms of landings (20–30% of total weight) and revenue for many years[1] (Ralston and Polovina, 1982; WPRFMC[2]). Research on adult *P. filamentosus* has included studies on sexual maturity and growth (Ralston and Miyamoto, 1983; Kikkawa, 1984; Okamoto[3]), trophic ecology (Parrish, 1987; Haight et al., 1993); distribution (Kami, 1973; Moffitt, 1980); habitat (Ralston et al., 1986; Moffitt et al., 1989); and mortality (Ralston, 1987). Relatively few larval and pelagic juvenile specimens of opakapaka have been collected; therefore, little is known of their early life history (Leis, 1987).

Exploratory research on recruited, epibenthic juvenile *P. filamentosus* has been conducted by the Honolulu Laboratory of the National Marine Fisheries Service (NMFS) since 1988. This has included initial habitat description[4] and work on age and growth of juveniles at Kaneohe Bay, Oahu (Parrish, 1989; DeMartini et al., 1994). Recent work has focused on evaluating techniques for assessing the distribution and abundance of juvenile opakapaka in Hawaiian waters. Characterizations of juvenile opakapaka abundance based on rod and reel, handlines, and bottom trawls, however, are either biased, imprecise, or destructive of habitat.[5]

[1] Kawamoto, K. E. 1992. Northwestern Hawaiian Islands bottomfish fishery, 1991. Southwest Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Honolulu, HI 96822–2396. Southwest Fish. Sci. Cent. Admin. Rep. H-92-12, 20 p.

[2] WPRFMC (Western Pacific Regional Fisheries Management Council). 1992. Bottomfish and seamount groundfish fisheries of the Western Pacific region, p. 57–71. 1991 Annual Report for the WPRFMC, Honolulu, HI 96813.

[3] Okamoto, H. Y. 1993. Project to develop opakapaka (pink snapper) tagging techniques to assess movement behavior. Sept. 1, 1990 to Aug. 31, 1992. Final Rep. of HI. Dep. Land and Nat. Res. (HDLNR) to NOAA., Award no. NA90AA-D-IJ466. HDLNR, DAR, Honolulu, HI 96814, 18 p.

[4] Moffitt, R. B., and F. A. Parrish. In review. Habitat use and life history of juvenile Hawaiian pink snappers, *Pristipomoides filamentosus*. Bull. Mar. Sci.

[5] Ellis, D. M., E. E. DeMartini, and R. B. Moffitt. 1992. Bottom trawl catches of juvenile opakapaka, *Pristipomoides filamentosus* (F. Lutjanidae), and associated fishes, *Townsend Cromwell* cruise TC-90-10, 1990. Honolulu Lab., Southwest Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Honolulu, HI 96822-2396. Southwest Fish. Sci. Cent. Admin. Rep H-92-03, 33 p.

Still and video cameras (baited and unbaited) have been used to sample other marine habitats, e.g. to compare data collected with other methods and to reduce biases associated with remote collection techniques (Uzmann et al., 1978; Grimes et al., 1982; Matlock et al., 1991; Moffitt and Parrish, 1992), and to study the natural history and estimate the abundance indices of different species (Miller, 1975; Priede et al., 1990; Auster et al., 1991; Armstrong et al., 1992; Michalopoulos et al., 1992). Baited video systems have some inherent biases (such as loss of odor attractant (or bait; Isaacs and Schwartzlose, 1975), and intra- or inter-specific competition for bait (Armstrong et al., 1992). Towed video systems and remotely operated vehicles (ROV's) have been used to collect analogous data without the use of bait. A towed system or ROV can sample a known area and assess organismal densities directly within that area. Although a towed and unbaited video system might be preferable if the objective is to estimate absolute fish density, we chose a stationary, baited system both in order to minimize the disturbance of bottom habitat and to provide an index of relative abundance similar to catch per unit of effort (CPUE) that would be sufficient for temporal comparisons.

In this paper, we assess the precision, accuracy, and efficiency of a baited video camera system for producing indices of fish abundance based on a visual record, and we compare these video data with a more traditional abundance index of longline CPUE. We first compared the two methods by pair-sampling during an initial cruise in 1992. Preliminary estimates of video precision were made with these data. Power and sample sizes for video sampling were again estimated with data obtained during 1993. Effects of station and depth for the 1993 cruise were also examined. We emphasize data for the target species, opakapaka, but include complementary data for Bleeker's balloonfish ("puffers"), *Torquigener florealis*, because of its numerical dominance in both types of samples.

Materials and methods

Sampling

Simultaneous longline and video camera operations were conducted for four days offshore of windward Oahu embayments (Fig. 1) and three days offshore of embayments at each of two other islands (Maui and Kauai) during a May 1992 cruise of the NOAA ship *Townsend Cromwell*. Additional video camera operations were conducted from small craft during May 1993 outside Kaneohe Bay, Oahu (Fig. 1). Sampling was conducted on flat, unconsolidated bottoms (Parrish, 1989; Moffitt and Parrish[4]) between 0800 and 1530 hours. Longline depths ranged from 54 m to 107 m and video camera depths ranged from 52 m to 87 m for the 1992 cruise, the approximate depth range for juvenile opakapaka (Parrish 1989; Ellis et al.[5]). Paired video-longline samples were collected in 1992 over a longshore spatial scale of 10 nmi (18.5 km) off Oahu (Fig. 1). The longshore spatial scales for Kahului Bay, Maui, and Hanalei Bay, Kauai, were 9.1 nmi (16.9 km) and 9.5 nmi (17.6 km), respectively. From one to three video deployments were made per station at shallow, mid-, and deep depths along the longline set. A total of 39 longline and video stations were completed (15 at Oahu, and 24 combined for Maui and Kauai). A complete set of three (shallow, mid-, and deep) video camera deployments were completed per station for 10 of the 15 stations off Oahu. In 1993, video sampling was refocused on a finer spatial scale (2.4 nmi or 4.4 km) off Kaneohe Bay, Oahu, over known bottom topography to increase the probability of sampling more homogeneous habitat. Two video deployments per station were made at shallow (73 m to 77 m) and deep (83 m to 85 m) positions in 1993. A total of 18 stations were completed in 1993.

Video camera and longline stations were parallel within 50 to 75 m of each other. The spacing between video-longline stations during any particular day was approximately 1 km (0.5 nmi) longshore during 1992. Spacing between video deployments was approximately 100 to 300 m. Longshore distance between video stations in 1993 was about 200 m (0.1 nmi). At distances of ≥100 m separating adjacent stations, successive video deployments were likely independent, because the greatest distance of fish attraction to the bait was only 48 to 90 m. This estimate was based on average maximum bottom current speeds of 0.1 to 0.2 m/s respectively (Bathen, 1978), a soak time of 10 minutes, and a swimming speed for opakapaka of 0.6 m/s (or approximately 3 body lengths (BL) per second, where one BL=20 cm; Videler, 1993). Depths of all video and longline sets were determined by depth sounders aboard the research vessels, and positions were determined by GPS (Global Positioning System) or sighting compass, as Loran-C capabilities were unavailable.

Longlines were deployed approximately perpendicular to depth contours. Bottom longline operations used modified Kali longlines,[6] each with 30 individu-

[6] Shiota, P. M. 1987. A comparison of bottom longline and deep-sea handline for sampling bottom fishes in the Hawaiian Archipelago. Honolulu Lab., Southwest Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Honolulu, HI 96822-2396. Southwest Fish. Cent. Admin. Rep. H-87-5, 18 p.

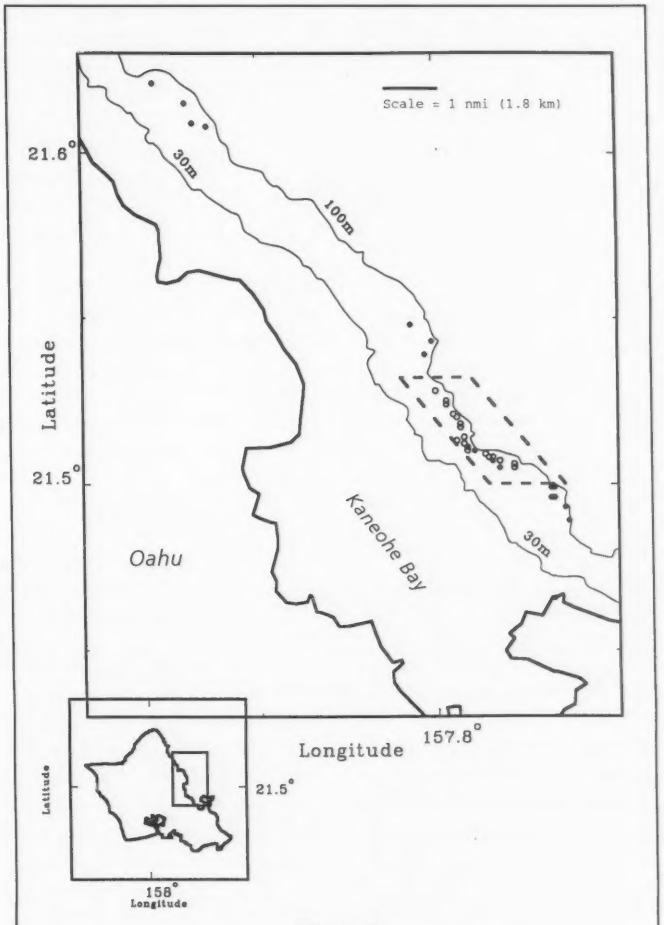

Figure 1

Area of operations off Kaneohe Bay, Oahu, for video and longline surveys for opakapaka, *Pristipomoides filamentosus*. Stations for video and longline in 1992 are midpoints identified by solid circles. Area of video coverage in 1993 is enclosed within the dotted lines and stations are midpoints identified by hollow circles.

ally weighted and buoyed 3-m PVC droppers. Droppers were attached along the main line about 18 m apart. A 9.07-kg test, hard monofilament branch leader and a 3.63-kg test, hard monofilament hook leader were used. Each dropper had five leaders with size–12 Izuo circle hooks (AH style), for a total of 150 hooks per longline set. Stripped squid was used as bait. The standard soak time was 30 minutes, and three to four sets were completed each day.

Two separate, 8-mm video camera assemblies were used for the video operations. Each video camera was equipped with a No. 1 diopter magnification lens and a wide angle zoom lens with a red filter for underwater correction. Camera focus, sensitivity, and white balance were manually adjusted, but an automatic aperture setting was used. The focus distance for both video cameras was fixed at 2.13 m, and the focal length of the lens was set at 11 mm. Each video camera was enclosed in an underwater housing and secured in a weighted frame (Fig. 2). A single, 15-cm long bait container was positioned 60 cm in front of the camera lens and mounted on a PVC rod. The bait container held a single (≈0.5-kg) mackerel (*Scomber* sp.) and one whole squid (*Loligo* sp.) tie-wrapped to the outside, both of which were changed after each deployment. The camera assemblies were manually

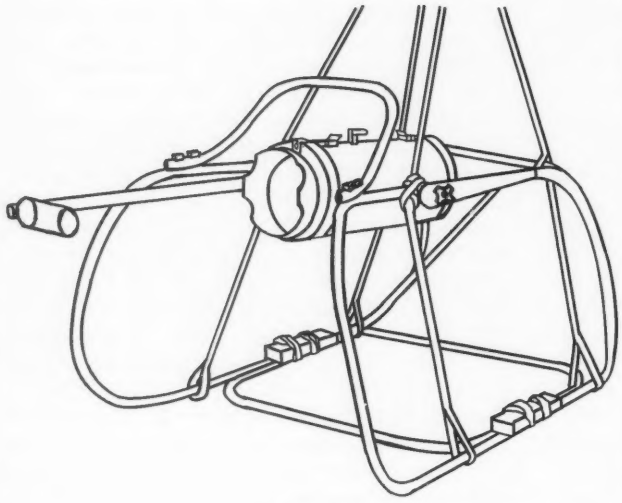

Figure 2
Baited video camera assembly with bait container positioned 60 cm in front of the camera lens.

lowered to the bottom and marked by a buoy; later they were raised to the surface by outboard engine power. Cameras were allowed to rest on the bottom for a standard interval of 10 minutes before retrieval.

The duration and number of video camera deployments to be used on the ship cruise were estimated on the basis of three earlier pilot deployments of the video assembly from small craft. These prior tests indicated that about 10 minutes were required to deploy and retrieve the camera assembly. The time to first appearance (TFAP) of opakapaka from the three pilot stations was 227 ± 300 sec (mean ± 1 standard deviation of the data [SD]) after bottom contact. A bottom time of 10 minutes was chosen to accommodate likely extremes and also to allow 6 deployments per 2-h tape (20 min per deployment × 6 deployments). With two cameras, 12 deployments per day could be made without changing tapes. The maximum number of longline sets was limited to four per day, based on three camera deployments per longline set.

Types of data

Species presence, total number of individuals per species, and the number of hooks lost were recorded for each longline set. Species presence and duration of squid bait attachment to the bait container (BTM) were recorded for each video sequence. In addition, for each video sequence, three indices of abundance were scored for each species taped: maximum number (MAXNO); time to first appearance (TFAP); and total duration in sequence (TOTTM). The MAXNO index was determined as the peak number of a species visible at any one time (maximum interval one second) during a deployment. Fork length (FL) to the nearest 0.1 centimeter (cm) was recorded for fish caught on the longline. The FL of opakapaka observed on video was estimated and rounded to the nearest 5 cm by comparing fish swimming in the plane of the bait container with the known size of the container.

Statistics

An average maximum number of fish recorded for data collected in 1992 was calculated for each of nine sequences (three video stations) by using a mean weighted by the duration of each occurrence:

$$\overline{X}_W = \frac{\sum_{h=1}^{n} X_h \cdot N_h}{N}, \quad (1)$$

where $\overline{X}_W$=the weighted average maximum number of fish, n=total number of occurrences, X_h= maximum number of fish seen in the h^{th} occurrence, N_h=duration (s) of the h^{th} occurrence, and $N=\sum N_h$= 600 s.

Video indexes were calculated as means (of up to 3 deployments) to standardize for multiple deployments per station. Video indices were derived in two logarithmic forms—mean of logs (*ML*),

$$ML = \frac{\sum_{i=1}^{n} \ln(x_i + 1)}{n}, \quad (2)$$

and log of means (LM),

$$LM = \ln\left[\left(\frac{\sum_{i=1}^{n} x_i}{n}\right) + 1\right], \quad (3)$$

where x_i = individual datum for a variable (i.e., the value for the variable MAXNO, TFAP, or TOTTM for each deployment at a station) and n=number of deployments per station. The longline index consisted of log-transformed individual set data [ln (catch +

1)]. The number of stations where each species was caught or seen was also tallied for each gear type. For nonzero mean data collected in 1993, the best form of the video index (*LM'*, see Results section), was calculated as follows:

$$LM' = \ln\left[\left(\frac{\sum_{i=1}^{n} x_i}{n}\right)\right], \quad (4)$$

where x_i = individual datum for MAXNO and n=number of deployments per station.

A matrix of Pearson's correlation coefficients was calculated for 1992 data (SAS, 1987) with the log-transformed variables to detect interrelationships among all the video and longline indices. Spearman's rank correlations were also calculated and compared. Multiple linear regression (SAS, 1987) was used to estimate the effect of competition between opakapaka and puffers for longline hooks on the basis of the following model:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \varepsilon, \quad (5)$$

where Y=ln (opakapaka video MAXNO), X_1=ln (no. hooks lost + no. puffers caught), and X_2=ln (number of opakapaka caught). The model was run as a forward regression without an intercept and with an entry level for significance equal to $P \leq 0.10$. The precision (repeatability) of video and longline was described by the coefficient of variation (V, Sokal and Rohlf, 1981; Zar, 1984):

$$V = \left(\frac{SD}{\text{mean}}\right) \times 100(\%), \quad (6)$$

where SD is the standard deviation.

Longshore station and relative depth effects for 1993 data were analyzed by using standard parametric and nonparametric procedures (SAS, 1987).

Sample size and power analysis

We evaluated video and longline data in a power analysis for the t-test of means. Specifically, we estimated the sample sizes required to detect a twofold change in abundance by using either sampling method. Skalski and McKenzie (1982) set a precedent for use of the criterion of twofold change in environmental monitoring studies; annual variations much larger than this are typical for marine fishes (Hennemuth et al., 1980; Francis, 1993). The effect size (ES) was calculated as follows:

$$ES = \frac{0.693}{SD}, \quad (7)$$

where 0.693= $|\pm$ twofold difference in $\bar{x}|$ for the natural log ($\bar{x}$) and SD is the standard deviation. Cohen (Tables 2.3.4 and 2.4.1, 1988) was consulted for the requisite sample sizes. The ES for each gear was evaluated at ß=0.20, power (1–ß)=0.8, and α_2=0.05. For the 1993 data, ES was also evaluated at α_2=0.1.

Results and discussion

Sample composition

The mean time to first appearance (TFAP) of opakapaka for 1992 video tapes with opakapaka present (all islands included) was 203 ±165 (SD) seconds. The total time (TOTTM) of opakapaka during a deployment averaged 122 ± 133 seconds. The maximum number (MAXNO) of opakapaka appeared on tape at approximately 354 (±153) s, based on the nine video sequences for which the weighted average MAXNO ($\bar{X}_W$) was calculated. These data confirm our initial choice of a 10-min bottom time.

In 1992, only windward Oahu data were used for comparisons and statistical analyses, because the opakapaka measures from Maui and Kauai included large percentages (92% and 67%) of "double-zeros" (zero longline catch, zero fish recorded). Catches of *P. filamentosus* also were greatest for the windward Oahu site; 54 of the 58 juvenile opakapaka were longlined off windward Oahu. Puffers were prevalent at windward Oahu and at Maui. Both longlined and video-recorded opakapaka were juvenile size (13 to 21 cm FL, and 15 to 25 cm FL, respectively; Kikkawa, 1984; Moffitt and Parrish[4]).

Frequency of occurrence data and total number of species differed between longline catches and video records (Fig. 3). Puffers ranked first in abundance and opakapaka second in both the longline and video data. Video cameras recorded the presence of opakapaka and puffers more often than did the longlines (Fig. 3). Video tapes also recorded a greater diversity of species (Table 1), suggesting greater accuracy of the video system. Fish that were not caught by the longline but were seen on video included reef-associated species (e.g. pennant butterflyfish, *Heniochus diphreutes*, and whitesaddle goatfish, *Parupeneus porphyreus*, sharks (*Carcharhinus* sp.), and rays (*Dasyatis* sp.). Longlines also undersampled the lizardfish, *Trachinocephalus myops* (Fig. 3), a major component of this deep-water, soft-bottom fish assemblage.[5] No major differences in species composition occurred in video surveys from 1992 and 1993.

1992 video-longline relations

The MAXNO index for opakapaka and puffers was highly correlated with the total duration on film

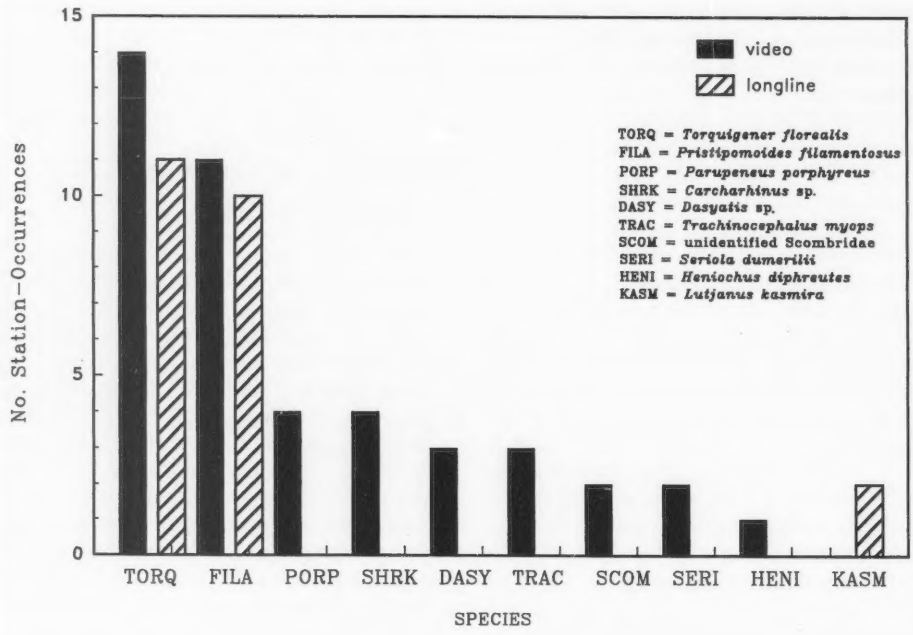

Figure 3

Frequency of occurrence of ten common species on video camera deployments and longline sets conducted during 1992 off windward Oahu (n=15 stations). Refer to Table 1 for common names.

Table 1

Total numbers of fish seen and caught at 15 video and longline stations located off windward Oahu during 1992. Total number for a fish taxon for video stations is the sum of the maximum numbers seen on 38 films. Total number for longline stations is the number of fish caught.

Species	Common names	Video	Longline
Torquigener florealis	Bleeker's balloonfish	221	80
Pristipomoides filamentosus	Pink snapper (opakapaka)	94	54
Heniochus diphreutes	Pennant butterflyfish	25	—
Parupeneus porphyreus	Whitesaddle goatfish	10	—
unidentified Scombridae	Tuna or mackeral	6	—
Trachinocephalus myops	Lizardfish	5	—
Carcharhinus sp.	Shark	4	—
Sphyrna sp.	Hammerhead shark	4	—
Seriola dumerilii	Amberjack	3	—
Dasyatis sp.	Stingray	3	—
Chaetodon miliaris	Milletseed butterflyfish	3	—
unidentified teleosts	Bony fish	2	—
Parupeneus pleurostigma	Sidespot goatfish	1	—
Sufflamen fraenatus	Bridle triggerfish	1	—
Canthigaster rivulata	Maze toby	1	—
Parupeneus sp.	Goatfish	1	—
Lutjanus kasmira	Bluestripe snapper	—	3

(TOTTM) and time to first appearance (TFAP) of the respective species (Table 2, LM form). The duration of squid bait (BTM index) was significantly correlated with the MAXNO index and the other video indices for opakapaka but was more strongly correlated with the MAXNO index for puffers (Table 2). Videos indicated that puffers were usually responsible for the removal of the squid bait; a direct relationship between puffer numbers and the rate of bait disappearance was evident. Spearman's rank correlations mirrored the parametric correlations.

After log-transformation, the data pairs were approximately bivariate normal. Among all the video indices, MAXNO was best correlated with lnCPUE (LLNO) for opakapaka (Table 2). The ML and LM forms of the MAXNO video index were compared separately with the longline CPUE, and the LM form provided a slight but consistently better Pearson's correlation than did the ML form for both opakapaka and puffers. Therefore, the LM form of the MAXNO index was used for all further parametric comparisons and analyses.

The MAXNO-CPUE relationship was approximately linear (Fig. 4A), and its residual plot showed neither discernible pattern nor slope (P=1.0, Fig. 4B). If all double-zero data are deleted, the correlation between video MAXNO and longline CPUE loses significance (r=0.55, P=0.08, n=11). However, the double-zero data were retained in subsequent analyses because there was no a priori reason to believe they did not represent real absences.

The observed magnitude of hook loss ($\bar{x}$=32%) indicates that longline CPUE is fundamentally inaccurate and biased for sampling this habitat and species assemblage. Apparently, most hook loss occurred when puffers bit through the leader above the hook. Hook competition is often a problem with longlines when hooked fish begin to saturate available hooks (Rothschild, 1967). Removal of hooks has a similar effect. A multiple linear regression with two descriptive variables, a puffer factor (X_1) equal to the number of hooks lost plus puffer catch and opakapaka catch (X_2), was run to determine the effect of puffers on the relation between longline CPUE and the video MAXNO index for opakapaka. X_1 and X_2 were first determined to be uncorrelated (r^2=0.02, P=0.62). The model (Eqn. 5) for the multiple regression was forced through the origin, because neither sampling device could record the presence of fish in its absence. The total variation in the opakapaka video index explained by the model was 87% (R^2=0.87, P<0.001). Opakapaka longline CPUE explained 83% of the variation (r^2=0.83, P<0.001), and the puffer factor explained an additional 4% of the variation (r^2=0.04, P=0.07). The latter observation suggests that the puffer factor might strongly influence video-longline relations for opakapaka at times of relatively high puffer abundance.

Precision for longline and video cameras was separately examined. For both opakapaka and puffers, cameras had nominally but consistently better precision (V=81% and 48%) than did longline CPUE (V=91% and 71%).

Table 2

Correlation between log-transformed mean video indices (LM) and log-transformed longline catch per unit of effort (lnCPUE) from the 1992 windward Oahu site (n=15 stations). Pearson correlation coefficients (r) are displayed above their respective P-values (Prob>|R|, H_o: Rho=0) for *Pristipomoides filamentosus* and *Torquigener florealis*. MAXNO = maximum number seen on tape; TOTTM=total duration of a species on tape; TFAP=time to first appearance of a species; BTM=duration of external squid bait; and LLNO=longline CPUE.

	TOTTM	TFAP	BTM	LLNO
Pristipomoides filamentosus				
MAXNO	0.9665	−0.9143	−0.5748	0.7855
	0.0001	0.0001	0.0250	0.0005
TOTTM		−0.8500	−0.5681	0.7285
		0.0001	0.0271	0.0021
TFAP			0.5729	−0.6467
			0.0256	0.0092
BTM				−0.2982
				0.2803
Torquigener florealis				
MAXNO	0.9465	−0.5770	−0.6654	0.5365
	0.0001	0.0243	0.0068	0.0392
TOTTM		−0.6030	−0.5902	0.5932
		0.0173	0.0205	0.0198
TFAP			0.5141	−0.1143
			0.0499	0.6851
BTM				−0.5193
				0.0473

1993 video statistics

The MAXNO video index did not differ between shallow and deep positions (Student's t=0.27, P=0.79; Kruskal-Wallis χ^2=0.09, P=0.76) in May 1993 (Fig. 5). The mean MAXNO data lack a monotone trend over stations (P=0.5), even though raw MAXNO values were atypically large at several stations (χ^2=28.0, P=0.04; Fig. 5). Overall, however, dependence among stations was absent and neither precluded simple t-tests of the means nor power estimates for tests of the means.

Power analysis

Power was estimated for opakapaka abundance indices. Sample sizes for longline and video gear would

be limited in practice by the duration and availability of a large research vessel (a maximum of five days at one location) and by estimates of maximum effort attainable for each gear. The latter considered the time needed for deployment, retrieval, and processing of specimens for longlines and the time required for video camera battery and tape changes. The estimated upper limit for sample size was 30 stations (at 6 stations per day) for both the longline and the video cameras; a video station consisted of three camera deployments. All 1992 data from windward Oahu (n=15 stations) were analyzed first. Sample sizes of 26 stations for video and 33 stations for longlines were estimated as necessary to detect a twofold change in numbers of juvenile opakapaka.

Power was next estimated by using only those 1992 windward Oahu stations with the full complement of three deployments (n=10). The variability (V=81%) of the video MAXNO index at these 10 stations (3 deployments inclusive) did not differ from the total Oahu data set. A sample size of 17 stations (51 deployments) was estimated as necessary to detect a twofold change in the MAXNO index, within the practical limit of 30 stations. The estimated sample size for longline, based on data for these same 10 stations, was still 33 stations, slightly over the limit of 30 stations.

Power was next reexamined to determine the effect of reducing effort to two video deployments per station, rather than to three. By using only the shallow and deep sets of the 10 1992 stations with three complete deployments, variability improved slightly (V=76%), and only 18 stations (36 deployments) were estimated as necessary to detect a twofold change for opakapaka. Video MAXNO remained significantly correlated with longline CPUE for opakapaka by using two deployments per station (r=0.66, P=0.04, n=10).

The 18 pairs of 1993 video data were used to reevaluate power and sample size for a smaller, more homogeneous study area. The LM' form of the video MAXNO index was used to avoid possible bias introduced by adding a constant to the data. The calculations used 17 pairs of deployments because the mean of one data pair was zero, the log of zero being undefined. Variability (V) for the 1993 data improved to 49% with these refinements. Twenty-two stations were estimated as necessary to detect a twofold change in abundance at α_2=0.05. If α_2 were relaxed to 0.1, 17 stations would be necessary to detect a twofold change. In practice, slightly greater numbers of samples would be necessary, because deleting the single zero-mean datum artificially inflated our power estimate. Nonetheless, the 1993 samples provide the best data for evaluating precision that are presently available.

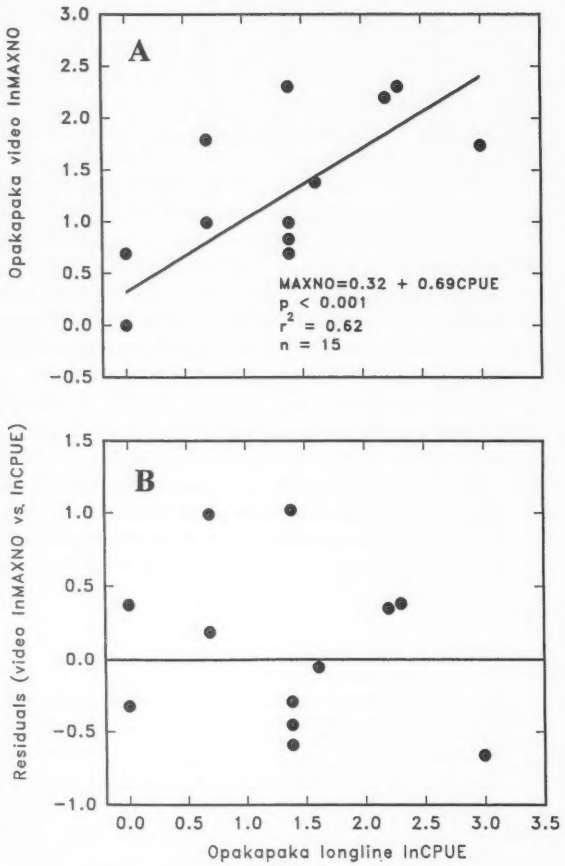

Figure 4

(**A**) Scatterplot and fitted regression line for the video index maximum number seen (lnMAXNO) and longline lnCPUE for *Pristipomoides filamentosus* (opakapaka) for 1992 data. Four double-zero observations were coincident. (**B**) Plot of residuals from the video versus longline regression of Fig. 4A versus longline lnCPUE for 1992 data; zero line is included. Four points were coincident as in 4A.

Comparison with other baited camera studies

Previous baited camera studies of deep-sea species (Priede et al., 1990; Armstrong et al.,

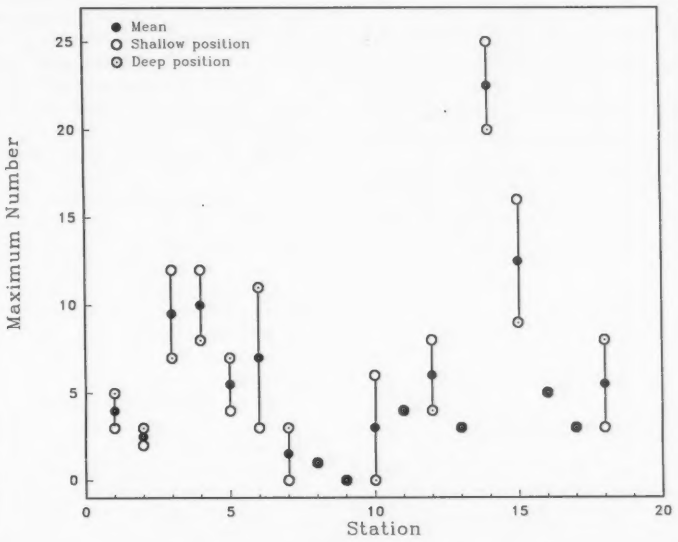

Figure 5

Scatterplot of maximum number of opakapaka observed (MAXNO) for shallow and deep positions and their mean by stations for data collected during May 1993. Stations are ordered in geographic sequence from farthest southeast (sta. 1) to farthest northwest (sta. 18). Means with component data hidden represent coincident shallow and deep values.

1992) observed that, although time of arrival of the first fish (TFAP) was strongly related to estimated fish densities, the maximum number of fish seen at a station (MAXNO) either was unrelated or inversely related to densities. Our observation that MAXNO was highly correlated with an abundance estimate (CPUE) may at first seem contradictory to these prior findings. However, there are important differences between our methods and those of previous studies: previous deep-sea work operated in unproductive depths >2,000 m and cameras recorded data for at least 11 hours per station, whereas our study was limited to productive depths ≤100 m for which a relatively short soak time (10 min) was sufficient. In the deep-sea studies, all bait was open to consumption. The partly internal bait of our system created a reservoir of odor that persisted for the soak duration in most cases; puffers removed all bait in only 3 out of 75 deployments. Differences in rates of bait consumption between the two deep-sea stations and resulting variations in bait attractiveness may have contributed to the disparity between MAXNO and fish density in the deep-sea studies.

The MAXNO and TFAP indices in our study were highly correlated (Table 2). This correlation suggests that the greater the density, the faster the fish arrive at the bait. These data agree with the observations of Priede et al. (1990), where fish arrived at the camera faster at the station with presumed higher densities. Since the MAXNO and TFAP indices were both significantly correlated with CPUE in our study (Table 2), the MAXNO index was chosen as the best index of abundance because it had the better correlation. A persistent bait source and short soak time may have contributed to this stronger correlation. In the future, the use of MAXNO as an index of abundance should be reevaluated separately for each species and application.

Conclusions

Video cameras provide an accurate tool for sampling juvenile opakapaka, and the video MAXNO variable provides a relatively precise and accurate index of abundance. Based on 1993 data for a series of two camera deployments per station, minima of 17 to 22 pairs of deployments (34–44 sets) per study area would be necessary to detect a twofold change in juvenile opakapaka numbers (at α_2=0.1, 0.05, respec-

tively). This could be accomplished within practical time limits (3–5 days) by using a large research vessel with two auxiliary small craft. If specimens are not needed—e.g. for age-growth studies—the video assembly described seems to be an ideal sampling technique for obtaining an index of abundance for comparisons between different areas or between different times, or between both. Additionally, video cameras can provide important, new information on habitat and behavior of juvenile opakapaka and associated species, an important aspect of our continuing research.

Acknowledgments

We would especially like to thank F. Parrish for development of a working video prototype and other assistance, S. Ellis for assistance in the reconfiguration of the video camera assembly, P. Shiota for assistance in modifying Kali longline gear, K. Landgraf for his help in the field with video gear, P. Shiota, T. Kazama, and D. Kobayashi for collecting longline sample data, and D. Yamaguchi for graphic assistance with Figure 2. We would also like to thank the officers and crew of the NOAA ship *Townsend Cromwell* for their assistance and cooperation during collection of data and R. Moffitt, W. Haight, S. H. Kramer, J. Parrish, and two anonymous reviewers for their helpful comments on the manuscript.

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Evolution of cytochrome *b* in the Scombroidei (Teleostei): molecular insights into billfish (Istiophoridae and Xiphiidae) relationships

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Abstract.—The suborder Scombroidei (Teleostei) has an extensive taxonomic history which traces its beginnings to at least 1832 (Cuvier and Valenciennes). However, a single well-corroborated phylogenetic hypothesis for the scombroid fishes does not exist. To date, efforts to define this suborder and determine the interrelationships of its constituent taxa have utilized morphological data almost exclusively. In this paper we present a molecular data set for addressing scombroid relationships: DNA sequences from the mitochondrial gene cytochrome *b*. These data provide valuable insights into scombroid relationships, especially regarding the long-standing debate over the placement of the billfishes (Istiophoridae and Xiphiidae). The cytochrome *b* data strongly refute a close relationship between Scombridae and billfishes and also support separation of the billfishes from the Scombroidei. In addition, these data suggest a new hypothesis on the evolutionary relationships among istiophorid billfishes.

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Fishery Bulletin 93:78–96 (1995).

The suborder Scombroidei is a well studied assemblage of more than 100 marine teleosts. A consensus on the taxonomic limits and intrarelationships of this group remains elusive despite more than 150 years of study (Cuvier and Valenciennes, 1832; Regan, 1909; Gregory, 1933; Berg, 1940; Fraser-Brunner, 1950; Collette et al., 1984; Johnson, 1986; Potthoff et al., 1986; Block et al., 1993). In 1832, Cuvier and Valenciennes proposed the Scombroidei as a natural group containing the oilfishes and snake mackerels (family: Gempylidae), the cutlassfishes (Trichiuridae), and the "tunnies" (Scombridae). Regan (1909) expanded the Scombroidei by adding three families: Istiophoridae (marlins, sailfish, and spearfishes); Xiphiidae (the swordfish); and Luvaridae (the louvar). Most subsequent classifications agree that the monotypic Luvaridae is not a scombroid but an acanthuroid (Leis and Richards, 1984; Tyler et al., 1989). However, there is substantial disagreement over the relationships of the families Istiophoridae and Xiphiidae, collectively known as billfishes.

In the last ten years three morphological studies have proposed three different hypotheses on the relationships of billfishes. In 1984, Collette et al. published a scombroid phylogeny based on 40 morphological characters (Fig. 1). They proposed that billfishes are the sister group of the Scombridae. Their cladogram suggested that several synapomorphies unite billfishes and scombrids, including a pharyngeal toothplate stay, a pair of lateral keels on the caudal peduncle, and the extension of the caudal-fin rays to cover the hypural plate. However, the position of billfishes was not strongly defined in the Collette et al. study because of homoplasious character evolution. Of the twelve character-state transitions that occurred within the billfish lineage on their cladogram, five were reversals to the primitive state, and six occurred independently in other lineages. Collette et al. (1984) considered the placement of billfishes within the suborder Scombroidei to be uncertain and conditional upon additional evidence. They cited larval evidence (Potthoff et al., 1986) which indicates that the scombroid families Scombridae, Gempylidae, and Trichiuridae are closely related to each other and are distantly related to billfishes. We will refer to the Collette et al. hypothesis as the scombrid sister group hypothesis.

In 1986, Johnson published a scombroid phylogeny using many of the characters from the Collette et

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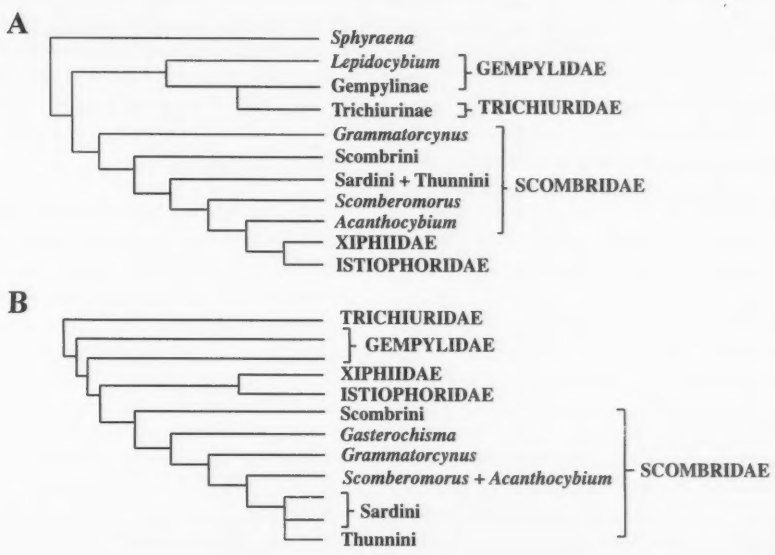

Figure 1

Two phylogenetic hypotheses for the scombroid fishes based on morphological evidence. The studies of (**A**) Johnson (1986) and (**B**) Collette et al. (1984) are examples of the scombrid-subgroup and the scombrid-sister group hypotheses of billfish (Istiophoridae and Xiphiidae) relationships. Johnson (1986) considered billfishes a subgroup of the family Scombridae most closely related to the wahoo, *Acanthocybium solandri*. Collette et al. (1984) placed the billfishes as a sister group to the Scombridae. Both hypotheses propose that billfishes and scombrids share a common ancestor to the exclusion of other scombroids. An alternative hypothesis for billfish relationships is that billfishes are not scombroids. This hypothesis has never been depicted explicitly in the form of a cladogram (Gosline, 1968; Nakamura, 1983; Potthoff et al., 1980; Potthoff et al., 1986).

al. (1984) study and several additional characters (Fig. 1). Like Collette et al., Johnson proposed that billfishes and scombrids compose a monophyletic group, but he regarded billfishes as a subgroup of the Scombridae. A critical piece of evidence supporting this hypothesis that billfishes are a derived group within scombrids is the presence of cartilaginous interconnections between gill filaments in billfishes and the scombrid *Acanthocybium solandri*. Based largely on this proposed synapomorphy, Johnson placed Istiophoridae and Xiphiidae as derived scombrids and *Acanthocybium* as their sister group. This association has been suggested by others (Lütken, 1880; Fraser-Brunner, 1950). However the position of billfishes in Johnson's study was only weakly supported because of homoplasy. For example, five of the ten character-state transitions that support billfish monophyly on Johnson's cladogram are reversals. We will refer to the Johnson hypothesis as the scombrid subgroup hypothesis.

Other workers have proposed that billfishes are not scombroids. In 1986, Potthoff et al. published a study of bone development in scombroids in which they discussed scombroid phylogeny. They concluded that billfishes are not scombroids because of their lack of resemblance to other scombroids in vertebral number and osteological development. They suggested that these characters indicate billfish affinities to the percoids. This hypothesis has been suggested in previous studies (Potthoff et al., 1980; Nakamura, 1983). We will refer to this hypothesis as the nonscombroid hypothesis.

It is evident from the morphological studies that there has been a great deal of homoplasious morphological evolution in billfishes. Therefore, it is difficult to reconstruct the evolutionary relationships of this group based on morphology alone. In an attempt to derive additional, independent data on scombroid intrarelationships and, in particular, to address the position of billfishes, we compiled a mo-

lecular data set that consists of DNA sequences from the mitochondrial gene cytochrome *b*. This gene codes for a functionally conserved protein that should facilitate sequence alignment over ancient divergences. Additionally, it has been used to examine both intraspecific genealogy (Finnerty and Block, 1992) and much deeper phylogenetic questions such as the origin of the mammalian orders (Irwin et al., 1991). The initial scombroid radiation probably occurred in the Paleocene epoch (Bannikov, 1985; Carroll, 1988). Therefore, cytochrome *b* sequence should be phylogenetically informative about divergences within the suborder.

The analysis presented in this paper builds on our earlier molecular study (Block et al., 1993). However, we have improved on the previous study in several ways which allow us to directly test the competing hypotheses of billfish relationships. First, we have obtained sequences from additional outgroups. The inclusion of presumably more distant outgroups permits us to address the question of scombroid monophyly. This is important because the nonscombroid hypothesis of billfish relationships argues that the Scombroidei is not a monophyletic group. Second, we include sequence information from the scombrid *Acanthocybium*, a taxon which is integral to the scombrid subgroup hypothesis. Third, we utilize statistical tests to directly compare different hypotheses of billfish relationships. Finally, we emphasize character-state changes that accrue relatively slowly in order to minimize the effects of phylogenetic noise.

Materials and methods

Samples

Partial cytochrome *b* sequences (590 base pairs) were obtained from 75 individuals representing 34 species of perciform fishes: 30 scombroid species and four putative outgroup taxa (*Sphyraena*, *Coryphaena*, *Mycteroperca*, and *Morone*; Table 1). We included *Sphyraena* based on the placement by Johnson (1986) of this taxon as the most primitive member of the Scombroidei. Several percoid taxa (*Coryphaena*, *Mycteroperca*, and *Morone*) were included because of the suggestion by some authors that billfishes are percoids (Gosline, 1968; Potthoff et al., 1980; Nakamura, 1983; Potthoff et al., 1986). Published cytochrome *b* sequences from two cypriniform fishes obtained from Genbank were used to root the phylogenetic analysis (*Crossostoma lacustre* [Tzeng et al., 1990] and *Cyprinus carpio* [Chang, 1994]). We verified the outgroup status of the cyprinids by first conducting a phylogenetic analysis using published sequence from the sturgeon *Acipenser transmontanus*, a holostean, to root a parsimony analysis. We attempted but were unable to obtain full length sequences (590 base pairs) from two fixed and preserved specimens of *Scombrolabrax heterolepis* possibly because of DNA degradation in these specimens.

DNA extraction

DNA was obtained from frozen tissue samples of the mitochondria-rich "heater tissue" (found in Istiophoridae, Xiphiidae, and *Gasterochisma melampus*; Block, 1986), red muscle, white muscle, or liver. Digestion of 0.1–0.6 g tissue was performed in ten volumes of extraction buffer containing 100 mM Tris Cl (pH 8.0), 10 mM EDTA, 100 mM NaCl, 0.1% SDS, 50 mM DTT, and 0.7 mg/mL proteinase K. Digestion proceeded for 2–4 hours at 41°C. The homogenate was extracted twice with equal volumes of phenol (pH 8.0), once with 1:1 phenol/chloroform, and once with chloroform. The final extract was precipitated with 1/9 volume of 3M sodium acetate (pH 5.2) and 2.5 volumes of 100% ethanol.

DNA amplification and sequencing

The polymerase chain reaction (PCR) was used to amplify a 700 base pair region of cytochrome *b*. A 305 base pair segment (not including primers) was generated by using published oligonucleotide sequences (Kocher et al., 1989). We amplified an overlapping, 425-bp region farther downstream with primers L15079 (5'-GAGGCCTCTACTATGGCTCTTACC-3') or L15080 (5'-CGAGGCCTTTACTACGGCTCTTACCT-3') and H15497 (5'-GCTAGGGTATAATT GTCTGGGTCGCC-3'). Double stranded amplification was performed in a 100-µL volume containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5–3.0 mM MgCl, 200 µM of each dNTP, each primer at 1 mM, 1 µg of template DNA, and 2 units of Amplitaq DNA polymerase (Perkin-Elmer/Cetus). Most templates were amplified through thirty cycles of PCR [1 minute denaturation (92–95°C), 1 minute annealing (40–50°C), and 3 minutes extension (72°C)] on an Ericomp thermal cycler. Alternatively, PCR was performed on a DNA Thermal Cycler 480 (Perkin-Elmer) with the following temperature cycling regime: 5 cycles of 1 minute denaturation at 95°C, 1 minute primer annealing at 40°C, 1:30 ramp to 72°C, and one minute extension at 72°C, followed by 25–35 cycles with an annealing temperature of 45°C. An 18-µL aliquot of the double stranded product was run by means of electrophoresis through a 1X TBE 1% agarose gel (Sea Plaque, FMC) at 5 V/cm for 45 minutes. A single stranded template was produced by asymmetric PCR (Gyl-

Table 1

Partial cytochrome b sequences (590 base pairs) were obtained from 34 perciform fishes, including 30 scombroid species. Published cytochrome *b* sequences were also obtained from Genbank for two cypriniform fishes.

Order and suborder[1]	Family and species	Common name	*n*	Locales[2]
Perciformes:Scombroidei	Istiophoridae			
	Istiophorus platypterus	sailfish	2	A, P
	Makaira indica	black marlin	2	I
	Makaira nigricans	blue marlin	8	A, P
	Tetrapturus albidus	white marlin	2	A
	Tetrapturus angustirostris	shortbill spearfish	2	P
	Tetrapturus audax	striped marlin	3	P
	Tetrapturus belone	Mediterranean spearfish	2	M
	Tetrapturus pfluegeri	longbill spearfish	1	A
	Xiphiidae			
	Xiphias gladius	broadbill swordfish	6	A, P
	Scombridae			
	Acanthocybium solandri	wahoo	3	A
	Scomberomorus cavalla	king mackerel	1	A
	Scomberomorus maculata	Spanish mackerel	2	A
	Gasterochisma melampus	butterfly mackerel	3	T
	Auxis thazard	frigate mackerel	2	P
	Euthynnus affinis	kawakawa	2	P
	Euthynnus alletteratus	little tunny	2	A
	Katsuwonus pelamis	skipjack tuna	2	P
	Thunnus alalunga	albacore tuna	2	P
	Thunnus albacares	yellowfin tuna	2	P
	Thunnus maccoyii	southern bluefin tuna	2	T
	Thunnus obesus	bigeye tuna	2	P
	Thunnus thynnus	northern bluefin tuna	2	A
	Sarda chiliensis	eastern Pacific bonito	1	P
	Sarda sarda	Atlantic bonito	2	A
	Scomber scombrus	Boston mackerel	2	A
	Scomber japonicus	chub mackerel	2	P
	Gempylidae			
	Gempylus serpens	snake mackerel	2	P
	Lepidocybium flavobrunneum	escolar	2	P
	Ruvettus pretiosus	oilfish	2	A
	Trichiuridae			
	Trichiurus lepturus	scabbard fish	3	A
Perciformes:Percoidei	Coryphaenidae			
	Coryphaena equiselis	pompano dolphin	2	P
	Serranidae			
	Mycteroperca interstitialis	yellowmouth grouper	1	A
	Percichthyidae			
	Morone saxatilis	striped bass	1	P
Perciformes:Sphyraenoidei	Sphyraenidae			
	Sphyraena sphyraena	Atlantic barracuda	1	A
Cypriniformes	Balitoridae			
	Crossostoma lacustre	hillstream loach	Tzeng et al., 1992	
	Cyprinidae			
	Cyprinus carpio	carp	Chang et al., 1994	

[1] Eschmeyer, 1990.

[2] A=Atlantic ocean; P=Pacific ocean; I=Indian ocean; T= Tasman sea; M=Mediterranean Sea.

lensten and Erlich, 1988) carried out in a 100-µL volume containing the same reactants as the initial PCR but using 10 µL of the dissolved gel band and reducing one primer concentration 100-fold. The product was washed by centrifugal dialysis with sterile water in Centricon microconcentrators (Amicon) to remove excess dNTP's. Sequencing was performed with the Sequenase kit (United States Biochemical, Cleveland, Ohio) by using the limiting primer from the asymmetric PCR reaction. Data from eight species were obtained by directly sequencing double-stranded PCR products. The template was purified prior to sequencing (either directly from the PCR reaction mix or following excision of the appropriate band from low-melt agarose) with Magic PCR Preps (Promega). Sequencing was performed with the Sequenase kit according to the specifications of Casanova et al. (1991). Sequences from *Mycteroperca* and *Morone* was obtained after first cloning the PCR products in pGEM t-vector (Promega) according to the manufacturer's instructions. Transformation was carried out by using XL–1 blue cells. Two positive clones were selected for each PCR product. Double-stranded sequencing (Sequenase 2.0) was performed following alkaline denaturation as recommended by the manufacturer. Sequence was obtained from both strands of the amplified fragment for all individuals.

Analysis

Sequences were aligned by using the MacVector program (IBI Biotechnologies). Maximum parsimony analysis was performed with PAUP 3.1. (Swofford, 1991). Neighbor-joining (Saitou and Nei, 1987) and UPGMA dendograms were constructed with Phylip 3.5 (Felsenstein, 1993). The strength of support for various nodes was assessed by using the bootstrap analysis (Felsenstein, 1985). Specific conditions for each analysis are contained in the figure legends.

Competing phylogenetic hypotheses were compared by using the "enforce topological constraints" option of PAUP 3.1. This option allowed us to determine the length difference between the most parsimonious trees that support each hypothesis. The cladistic permutation test for monophyly and nonmonophyly (Faith, 1991) was then used to ascertain whether the more parsimonious hypothesis is significantly better than the competing hypothesis according to the criterion of parsimony. The test was performed as follows. The actual length difference between trees supporting the two opposing hypotheses was obtained. Then 99 permuted data sets were constructed from the original data set by randomly shuffling the character states for each character. We then obtained the length difference between trees supporting the two opposing hypotheses for each permuted data set. If the actual length difference was matched or exceeded fewer than 5 times in all 100 data sets (the original data set plus 99 permuted data sets), then the more parsimonious hypothesis was considered to be significantly better than the less parsimonious hypothesis. This corresponds to a topology-dependent permutation tail probability, or T-PTP, of less than or equal to 0.05.

The effects of character weighting on parsimony analysis were assessed by EOR weighting (Thomas and Beckenbach, 1989; Knight and Mindell, 1993): each type of nucleotide substitution was weighted according to the ratio of its expected number of occurrences divided by its observed number of occurrences, or EOR. There are six types of nucleotide substitutions if we disregard the direction of change: A⇔G, C⇔T, G⇔T, G⇔C, A⇔T, and A⇔C. The observed number of each substitution type was obtained through pairwise sequence comparisons. Pairwise comparisons were performed between sets of sister species (sister species were identified through an initial unweighted phylogenetic analysis; see Fig. 2). Sister-species comparisons were used for two reasons. First, within a clade, sister species will tend to represent relatively recent speciation events. This recency lessens the chance that multiple substitutions have occurred at the same site and that more recent substitutions obscure older ones. Second, all comparisons between pairs of sister species are mutually independent. Therefore, if we restrict our comparisons to sister species, we cannot count the same base substitution twice.

We modified the method of Knight and Mindell (1993) to derive the expected number of substitutions in each class. This method accounts for differences in the frequencies of the four nucleotides that greatly influence the expected frequency of each substitution type. For instance, if guanine residues are very rare, then substitutions of other nucleotides for guanine will also be rare. The L-strand base composition of cytochrome *b* in scombroid fishes is strongly skewed (Table 2), as it is in other groups examined (for example, Irwin et al., 1991). Cytosines and thymidines each compose nearly 30% of the total nucleotide population whereas guanines compose less than 16%. In order to incorporate knowledge of the base composition into our derivation of the expected number of each substitution type, we proceeded as follows. First, the average frequency of each nucleotide (f) was obtained for all species used in the pairwise sequence comparisons. Second, the observed number of each substitution type ($S_{O[i \Leftrightarrow j]}$), where i and j represent two different nucleotides, was obtained by summing the results from all pairwise comparisons of

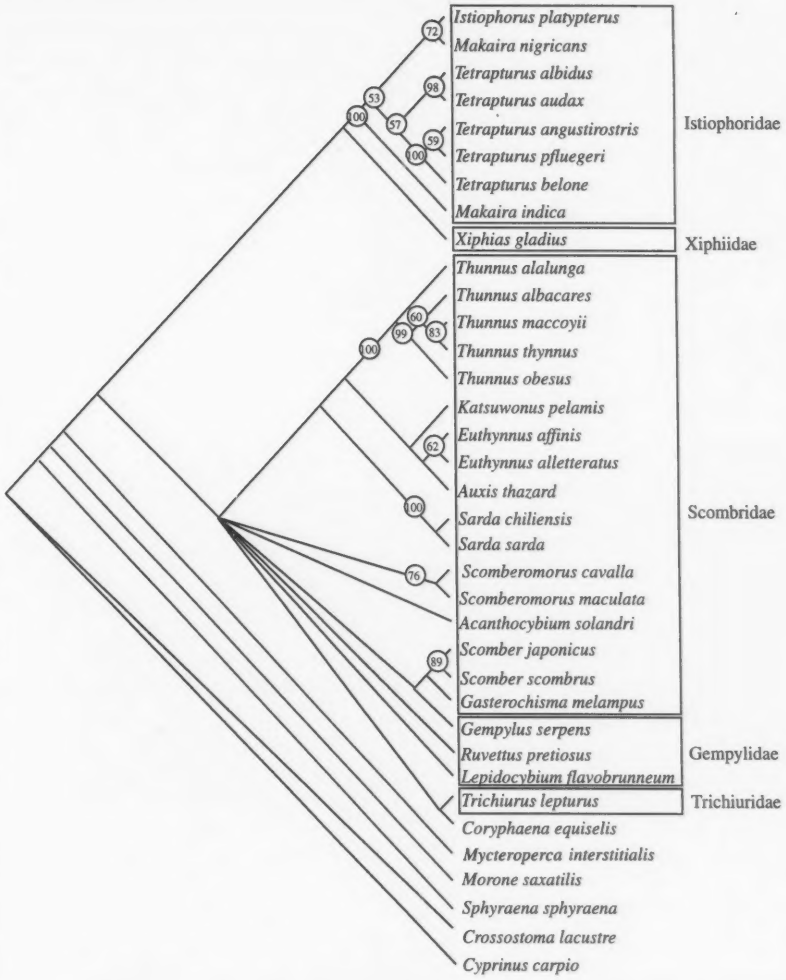

Figure 2

Phylogeny of the Scombroidei based on an unweighted analysis of 248 phylogenetically informative nucleotide sites. The cladogram depicted is a strict consensus of four equally parsimonious trees identified by using a heuristic search procedure on the program PAUP 3.1. (Swofford, 1991): TBR (tree bissection and reconnection) branch swapping was performed on 10 starting trees generated through random stepwise addition of taxa. *Crossostoma* and *Carpio* were specified as the outgroup. Length, consistency index, and retention index are the following: L=1595, CI=0.317, RI=0.539. Circled numbers at nodes indicated the percentage of trials in which a given partition between taxa is supported in 1,000 replications of the bootstrap analysis (Felsenstein, 1985). Only nodes supported in >50% of bootstrap replications are indicated.

sister species. The expected number of each of the six substitution types ($S_{E[i\Leftrightarrow j]}$)was then derived as follows:

$$S_{E[i\Leftrightarrow j]} = \left(f_i + f_j\right)\left(S_{0[total]}\right)/3.$$

We divide by three because three types of base substitutions are possible for each base, and we are interested in obtaining an expectation for one of them. For example, the expected number of A⇔T substitutions equals the average frequency of A's (0.23) plus

Table 2

Nucleotide substitutions by type determined through pairwise alignments. The observed substitutions for each type, $S_{O[total]}$, where i and j represent two different nucleotides, were calculated by summing the results from 8 pairwise comparisons of sister taxa. The expected substitutions for each type, $S_{E[total]}$, were calculated according to the formula $S_{E[total]}=(f_i+f_j)(S_{O[total]})/3$, where f_i and f_j are the frequency of nucleotides i and j. Average base frequencies for the 16 species are as follows: G=0.16, A=0.23, T=0.29, C=0.32.

	Substitution Types						
	TRANSITIONS				TRANSVERSIONS		
Pairwise comparison	A⇔G	C⇔T	G⇔T	G⇔C	A⇔T	A⇔C	Total
Tetrapturus audax vs. *Tetrapturus albidus*	0	0	0	1	1	0	2
Tetrapturus angustirostris vs. *Tetrapturus pfluegeri*	7	1	0	0	0	0	8
Makaira nigricans vs. *Istiophorus platypterus*	1	21	0	0	1	0	23
Euthynnus affinis vs. *Euthynnus alletteratus*	5	27	0	0	3	3	38
Thunnus thynnus vs. *Thunnus maccoyii*	4	3	0	0	1	0	8
Scomberomorus maculata vs. *Scomberomorus cavalla*	13	38	1	1	8	11	72
Sarda sarda vs. *Sarda chiliensis*	6	15	0	3	0	2	26
Scomber japonicus vs. *Scomber scombrus*	25	35	2	5	6	6	79
Total observed substitutions	61	140	3	10	20	22	256
Expected substitutions (see Methods section)	33.28	52.05	38.40	40.96	44.37	46.92	256
Expected/observed ratio (EOR)	0.55	0.37	12.80	4.10	2.22	2.13	

the average frequency of T's (0.29) multiplied by the total number of substitutions (256) divided by three, or 44.37 (Table 2).

The weights used for each substitution type (Table 2) are the ratios of expected substitutions divided by observed substitutions for that substitution type, rounded to the nearest integer (expected divided by observed ratios, or EOR's). All EOR's less than one were rounded to one. Weights were entered into PAUP 3.1. (Swofford, 1991) in the form of a step matrix.

Results

Sequence evolution and interfamilial relationships

Molecular data sets, such as the cytochrome *b* sequences presented in this study, are known to encompass subsets of characters that evolve at different rates. Subsets of data that differ in their evolutionary rates will also differ in their phylogenetic utility. Character state changes that accrue very rapidly should permit resolution of very recent divergences. However, these rapid character state changes can provide false inferences about distant relationships because of homoplasy. The likelihood of reversals and independent acquisitions is high if a particular site is evolving rapidly because there are only four possible character states (G, A, T, and C) and only six possible types of character state change (A⇔G, C⇔T, G⇔T, G⇔C, A⇔T, and A⇔C). Therefore, in order to make an accurate reconstruction of the earliest branching events in scombroid history, we should emphasize slowly evolving character state changes.

In an effort to best utilize the phylogenetic information from both slowly and rapidly evolving character state changes, our phylogenetic analysis proceeds in several discrete steps. We begin with an unweighted analysis of all informative nucleotide sites. This analysis is strongly influenced by nucleotide substitutions that accrue rapidly and should be most informative concerning recent speciation events. We then attempt to improve our resolution of more ancient divergences by giving greater weight to less frequent types of nucleotide substitutions. We conclude with a phylogenetic analysis based on the inferred amino acid sequences. The amino acid sequences evolve very slowly and should provide our most reliable estimates of the earliest splits between lineages. In each instance, the phylogenetic analysis is preceded by a discussion of the evolutionary variation in the character subset under consideration.

Unweighted nucleotide analysis

A 590-base pair fragment of the cytochrome *b* gene, representing positions 134 through 723 of the human cytochrome *b* sequence, was aligned across all

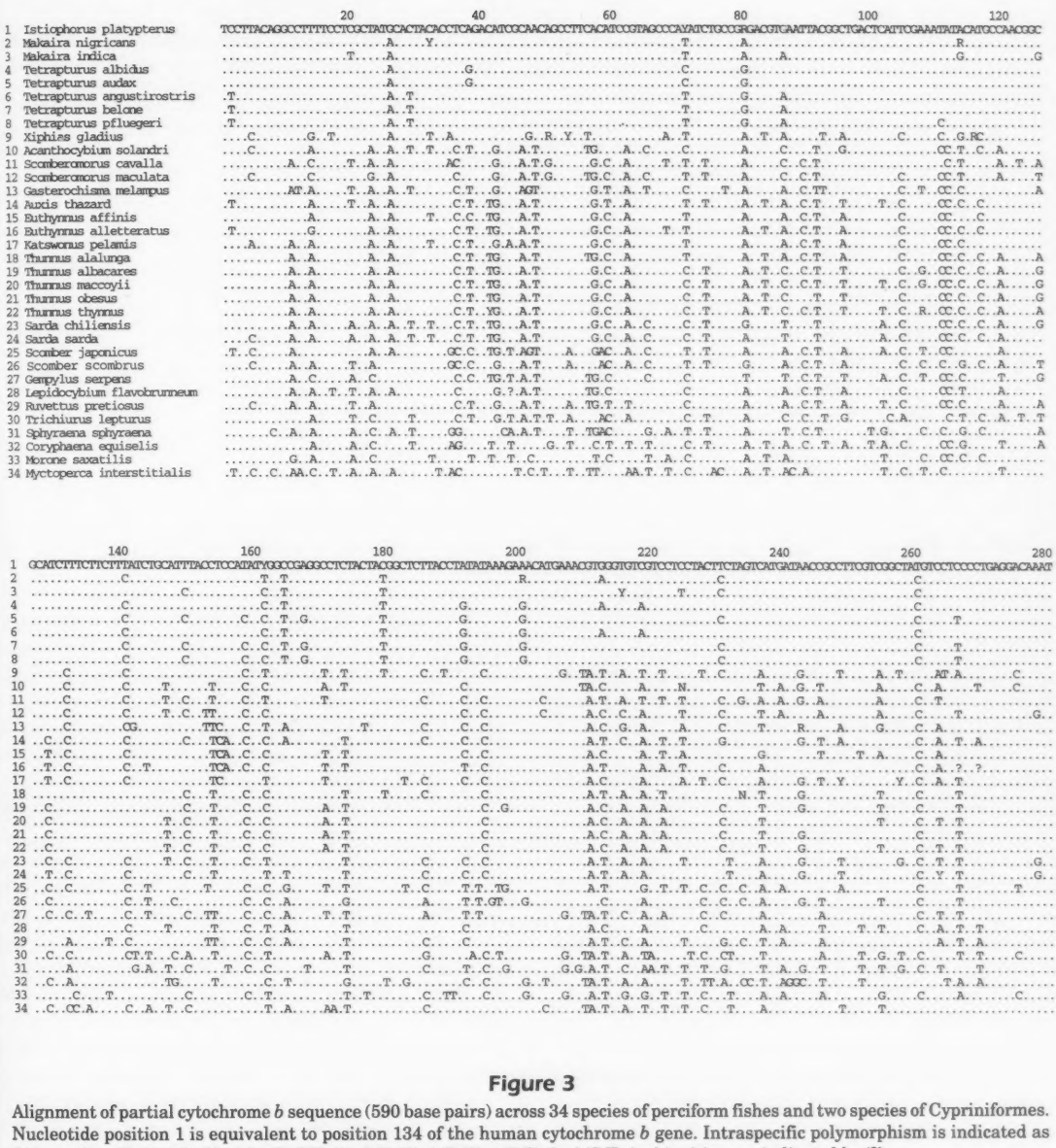

Figure 3

Alignment of partial cytochrome *b* sequence (590 base pairs) across 34 species of perciform fishes and two species of Cypriniformes. Nucleotide position 1 is equivalent to position 134 of the human cytochrome *b* gene. Intraspecific polymorphism is indicated as follows: R=A/G, Y=C/T, M=A/C, S=C/G, K=G/T, W=A/T, H=A/T/C, D=A/G/T. Ambiguities are indicated by '?'

thirty-six species included in the analysis (Fig. 3). No deletions or insertions were detected. Overall, 293 nucleotide positions are variable; 248 were potentially phylogenetically informative. As expected for a protein coding sequence, the degree of nucleotide variability differs according to codon position (Table 3). The third position is most variable and the second position is least variable. Differences in nucleotide variability at the three codon positions are due to the fact that many third position substitutions are silent, whereas many second position substitutions result in nonconservative amino acid replacements.

The differences in substitution rates between codon positions becomes more apparent when we compare

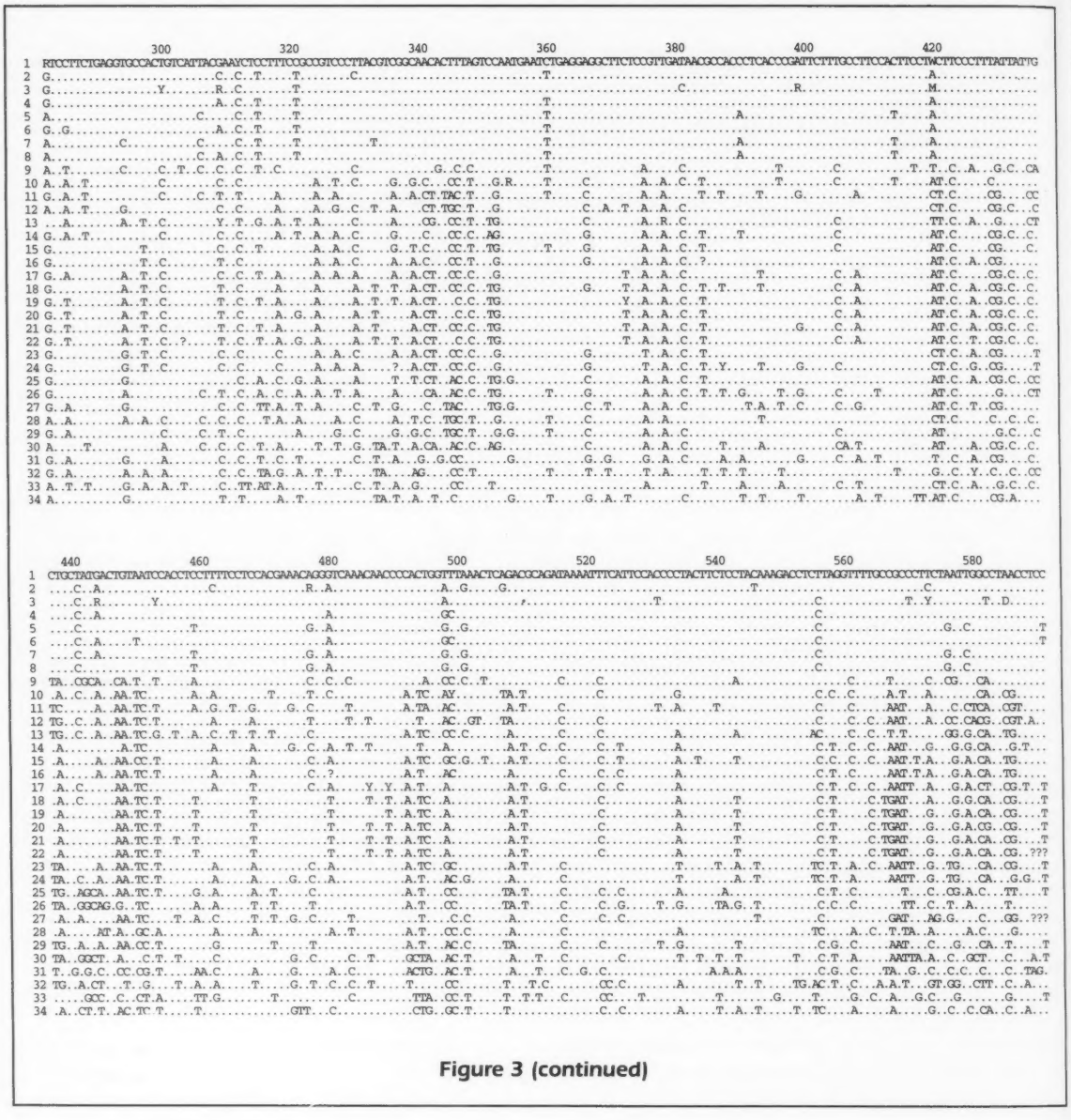

Figure 3 (continued)

the inferred number of substitutions (Table 3). For example, if a nucleotide site is twofold variable, i.e. if two bases occur at that position in an alignment of all species, then at least one base substitution has occurred at that position during the evolutionary history of the species concerned. Likewise, if a position is threefold variable, at least two substitutions have occurred, and so on. By this approximation, the 293 variable positions have experienced at least 521 substitutions, and substitutions at the third position outnumber substitutions at the first and second positions by nearly 4 to 1 and by more than 12 to 1, respectively.

Figure 2 presents a phylogeny of the Scombroidei based on an unweighted parsimony analysis of all informative nucleotide sites. In this cladogram, only the relationships among recently diverged taxa are strongly supported. There is support for the monophyly of genera within the family Scombridae (*Thunnus*, *Euthynnus*, *Sarda*, *Scomber*, and *Scom-*

Table 3

Variable sites in the cytochrome *b* nucleotide alignment (Fig. 2) according to codon position. Variable sites are further characterized according to how many nucleotide states are present: 2 states=twofold variable, 3 states=threefold variable, fourstates=fourfold variable.

	Codon position			
	1	2	3	Total
Total sites	196	197	197	590
Variable sites	72	29	192	293
A) twofold variable sites	50	27	69	146
B) threefold variable sites	15	2	49	66
C) fourfold variable sites	7	0	74	81
Minimum inferred substitutions = [(A) + 2(B) + 3(C)]	101	31	389	521
Phylogenetically informative variable sites	42	18	188	248

beromorus), and for the monophyly of the family Istiophoridae. Interrelationships within the family Istiophoridae and the genus *Thunnus* are well resolved. No other nodes are supported by more than fifty percent of bootstrap replicates (Felsenstein, 1985). Furthermore, there is a substantial polychotomy.

Weighted nucleotide analysis

The lack of resolution in the unweighted nucleotide analysis is not entirely unexpected. From Table 3, we can surmise that many nucleotide sites have incurred multiple substitutions and therefore the likelihood of convergent substitutions or reversals is high. In order to minimize the confounding effects of these homoplasious base substitutions, we have weighted infrequent substitution types more heavily using a modification of the method of Knight and Mindell (1993). If we disregard the direction of character change, we can place all nucleotide substitutions into six classes : A⇔G, C⇔T, G⇔T, G⇔C, A⇔T, and A⇔C. Through pairwise sequence comparisons we obtained observed counts for each of these substitution types (Table 2; also see Methods section). We observe a nearly 50-fold difference between the most common (C⇔T) and the least common (G⇔T) substitution types. Then, from the total number of observed substitutions and the observed frequency of each base, we derived the expected number of occurrences for each substitution type. The ratios of expected occurrences to observed occurrences for each substitution type (EOR's) were then used to weight the six types of base substitutions. The result of this weighting scheme is that substitution types that occur less frequently than expected are weighted more heavily.

A phylogeny based on EOR weighting of nucleotide substitutions is presented in Figure 4. It retains all of the strongly supported nodes that appear in the unweighted topology. In addition, the weighted topology contains three more basal nodes that are strongly supported by the bootstrap analysis (>50%): the node uniting Gempylidae, Scombridae, and Trichiuridae, the node uniting Xiphiidae and Istiophoridae, and the node uniting *Auxis* and *Euthynnus*. This suggests that the character weighting scheme has accomplished its goal to some extent: we have retained the phylogenetic signal from rapidly evolving substitutions while emphasizing the phylogenetic signal from slowly evolving substitutions.

According to the weighted cladogram (Fig. 4), all scombroids fall into two clades. The billfishes comprise one clade consisting of a monophyletic Istiophoridae and its sister group, Xiphiidae. All other scombroids (Gempylidae, Scombridae, and Trichiuridae) fall into a separate clade. This major split within the suborder Scombroidei is in agreement with our previous study (Block et al., 1993). However, in contrast with our previous study, the use of character weighting and the inclusion of more distant outgroups leads to the result that the suborder Scombroidei is not monophyletic. On the most parsimonious tree, *Sphyraena* and *Coryphaena* share a common ancestor with the gempylid-scombrid-trichiurid clade to the exclusion of billfishes, though this node does not receive particularly strong support from the bootstrap analysis. This result indicates some support for the hypothesis that billfishes are not scombroids. More importantly, the cladogram excludes the possibility that billfishes and scombrids comprise a monophyletic group within the Scombroidei, as required by the scombrid subgroup and scombrid sister group hypotheses. In summary, the weighted analysis agrees with the nonscombroid hypothesis and conflicts with the scombroid subgroup and scombroid sister group hypotheses.

Amino acid analysis

Amino acid substitutions occur far less frequently than nucleotide substitutions owing to the strong functional constraints on many regions of the molecule. Cytochrome *b* is a component of the electron transport chain and spans the inner mitochondrial membrane. The portion of the gene sequenced in this study encodes 195 amino acids corresponding to residues 46 through 240 of the human cytochrome *b* (Fig. 5).

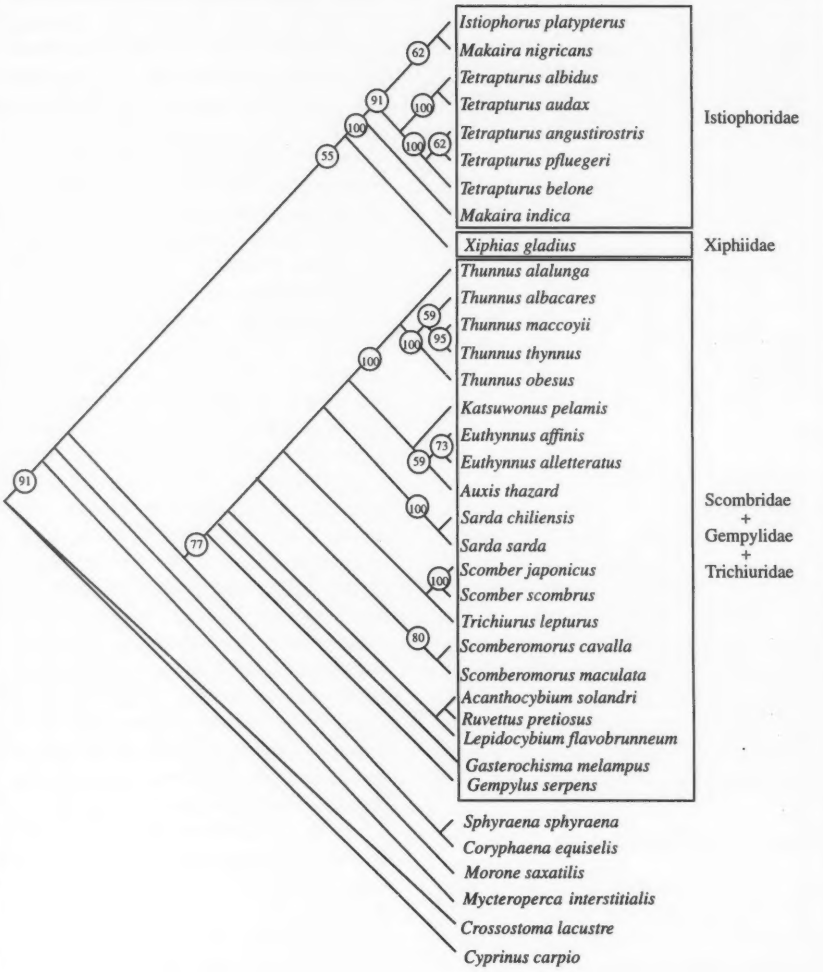

Figure 4

Phylogeny of the Scombroidei based on a weighted, maximum parsimony analysis of informative nucleotide sites. The six types of nucleotide substitutions are weighted according to the ratio of their expected occurrence to their observed occurrence (see Table 3). Weights used for each substitution type are the following: A⇔G=1, C⇔T=1, G⇔T=13, G⇔C=4, A⇔T=2, and A⇔C=2. *Crossostoma* and *Carpio* were specified as the outgroup. The tree depicted is the single most parsimonious topology identified in a heuristic search: TBR branch swapping was performed on 10 starting trees generated through random stepwise addition of taxa. Tree length is 2,348 steps. PAUP 3.1. was unable to derive consistency and retention indices for the cladogram that incorporated the weighting scheme. Circled numbers at nodes indicate the percentage of trials in which a given partition between taxa is supported in 1,000 replications of the bootstrap analysis (Felsenstein, 1985).

This fragment spans four transmembrane domains and includes part of the region implicated as the outer membrane redox reaction center (Howell and Gilbert, 1988; Howell, 1989; Fig. 6). In a comparison of the inferred peptide sequences across the 36 species included in this study, 134 (69%) of the 195 amino acid residues are invariant, 34 (17%) occur in 2 amino acid states, and 27 (14%) occur in 3 or more states.

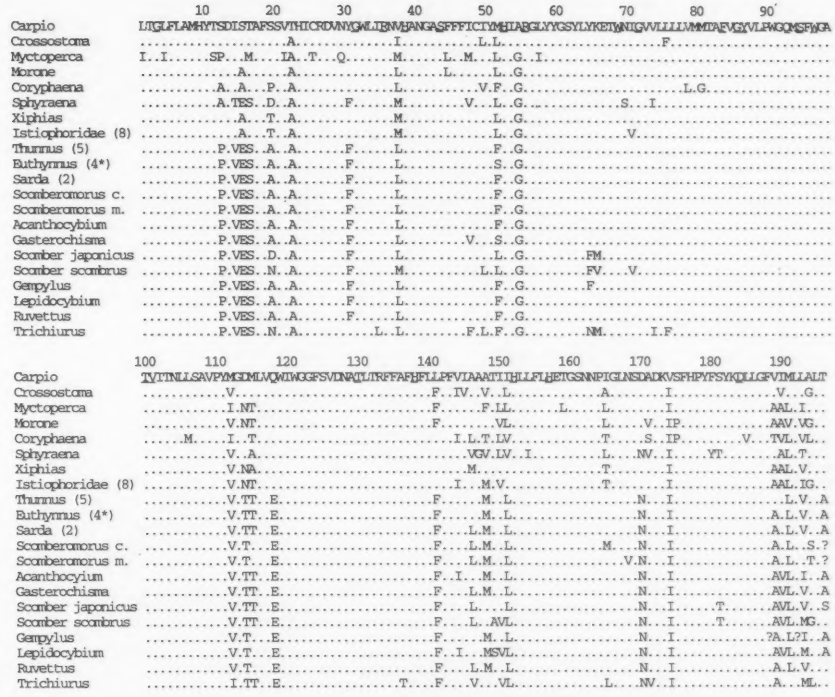

Figure 5

Alignment of inferred amino acid sequences. The amino acid sequences were inferred from the nucleotide sequences presented in Figure 3 by using the translation option of MacVector (IBI technologies) and the animal mitochondrial genetic code. There was no variation in inferred amino acid sequence within the family Istiophoridae, within the genus *Thunnus*, within the genus *Sarda*, nor among the genera *Auxis*, *Euthynnus*, and *Katsuwonus*. Conserved positions identified by previous studies are underlined in the query sequence, *Carpio* (Howell and Gilbert, 1988; Esposti et al., 1993).

This level of variability in amino acid sequence is very similar to that reported in a study of placental mammals, a group whose divergence times are probably comparable to scombroids (Irwin et al., 1991). Much of the variation in scombroid cytochrome *b* occurs in the transmembrane portion of the molecule and represents substitutions between hydrophobic residues (leucine, isoleucine, and valine). The largest stretches of invariant residues (21 and 17) occur in a region implicated as part of the Q_o redox reaction center (Howell and Gilbert, 1988; Howell, 1989; Fig. 6). All of the functionally constrained sites identified by previous studies are conserved throughout the fishes included in this study (see Fig. 5; Howell and Gilbert, 1988; Esposti et al., 1993).

Figure 7 presents a parsimony analysis based on 38 informative amino acid sites. The amino acid sequences do not provide information about more recent speciation events because they evolve very slowly, but they contain important evidence about the relationship of billfishes to other scombroids. The amino acid analysis shares two important similarities with the weighted nucleotide analysis: first, Scombridae, Gempylidae, and Trichiuridae comprise a clade, and second, *Sphyraena* and *Coryphaena* share a common ancestry with this Scombridae-Gempylidae-Trichiuridae assemblage to the exclusion of the billfishes (Xiphiidae and Istiophoridae). The node uniting *Sphyraena* with the scombrid-gempylid-trichiurid clade is one of the more strongly supported nodes according to the bootstrap analysis. Therefore, cytochrome *b* amino acid substitutions support the non-scombroid hypothesis and conflict with the scombrid subgroup and scombrid sister group hypotheses.

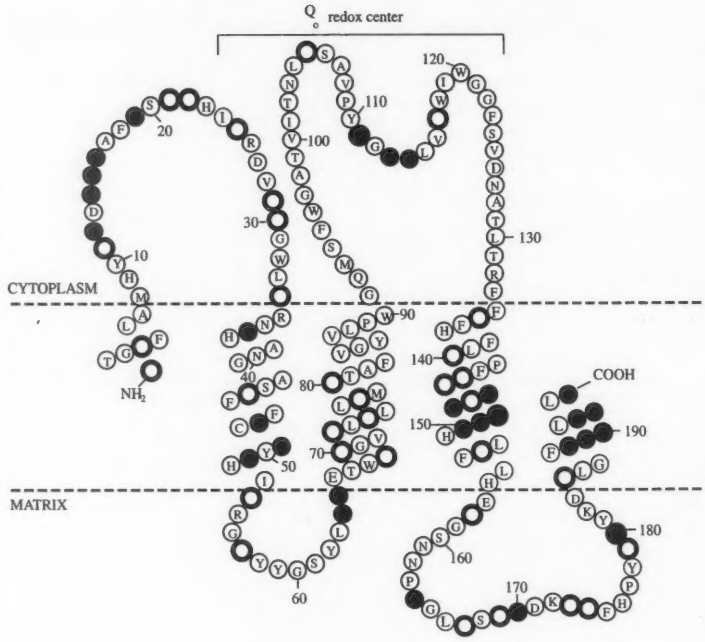

Figure 6

Variability in amino acid sequence superimposed over a structural model for cytochrome *b* (Howell, 1989). Hypervariable residues, present in three or more amino acid states, are indicated by solid circles. Variable residues, present in two states, are indicated by open circles. The amino acids present at invariant residues are specified on the diagram. Residue 1 of this fragment is equivalent to the forty-sixth residue from the amino terminal of the protein in humans.

The strength of the evidence that billfishes are not scombroids can be emphasized by directly examining the amino acid characters that are informative about this issue. Of the 38 informative amino acid sites, no sites unite billfishes and other scombroids to the exclusion of other perciforms, whereas eight sites unambiguously separate billfishes from all other scombroids, i.e. sites where all billfishes possess one character state and all other scombroids possess some other character state (characters 12, 14, 15, 16, 113, 117, 140, and 169; Fig. 5). At all of these sites, billfishes share the same character state as one or more of the percoid fishes. Furthermore, at three of these eight sites (15, 16, and 169), Gempylidae, Scombridae, and Trichiuridae share a common state with *Sphyraena* to the exclusion of all other species in the study. As this character analysis emphasizes, the amino acids are consistent with the hypothesis that billfishes are not scombroids and that *Sphyraena* is the sister group of a clade consisting of Gempylidae, Scombridae, and Trichiuridae.

Intrafamilial relationships

Within the family Istiophoridae (*Istiophorus*, *Makaira*, and *Tetrapturus*), cytochrome *b* nucleotide sequence provides a particularly well resolved and strongly supported phylogenetic signal. This is probably due to the recency of the istiophorid radiation. The maximum sequence divergence between any two species within this clade is less than five percent. We have performed a more in depth analysis of the interrelationships of istiophorids using the exhaustive search option of PAUP 3.1 (Swofford, 1991). Use of the exhaustive search option guarantees identification of the most parsimonious tree. The topology of this tree is identical to the topology of the istiophorid clade within the more inclusive scombroid phylogeny (Fig. 8, cf. Fig. 2). Neighbor-joining and UPGMA analyses produce an identical topology. Computer simulations suggest that agreement between these three methods should increase our confidence in a phylogenetic hypothesis (Kim, 1993).

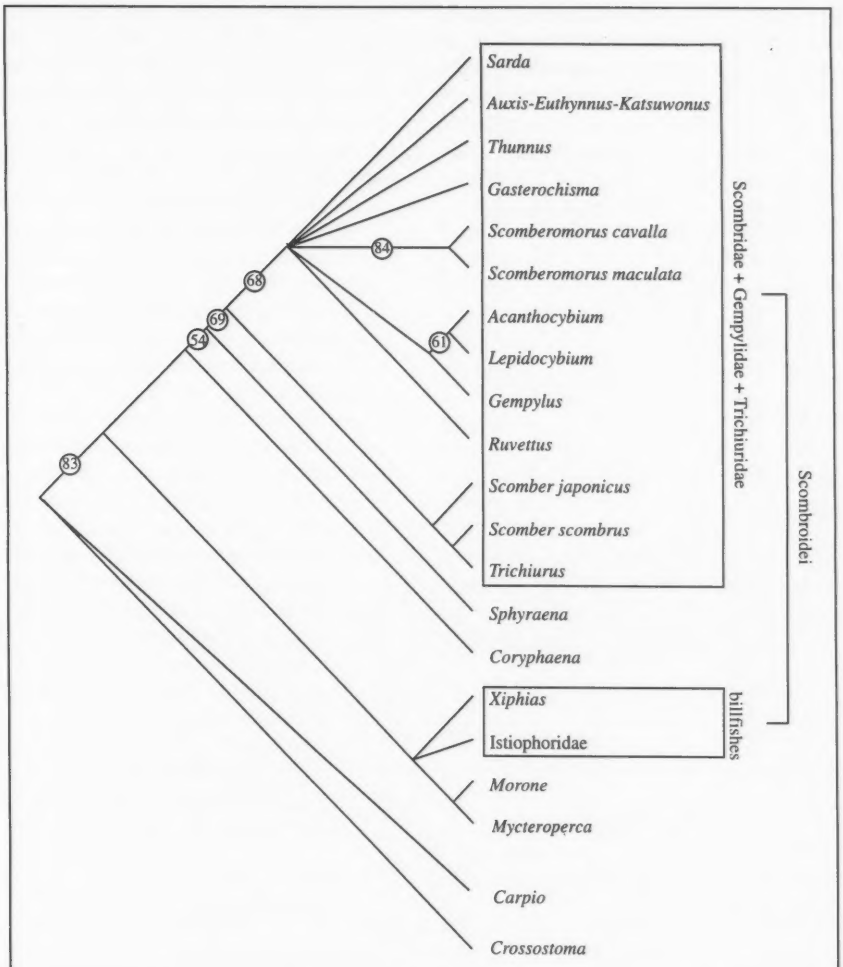

Figure 7

Phylogeny of the Scombroidei based on maximum parsimony analysis of inferred amino acid sequences. The cladogram depicted is a strict consensus of 68 equally parsimonious trees identified through a heuristic search procedure: TBR branch swapping was performed on 10 starting trees generated through random stepwise addition of taxa. *Crossostoma* and *Carpio* were specified as the outgroup. Length, consistency index, and retention index are the following: L=141, CI=0.567, RI=0.619. Circled numbers at nodes indicate the percentage of trials in which a given partition between taxa is supported in 1,000 replications of the bootstrap analysis (Felsenstein, 1985).

Cytochrome *b* sequence strongly supports the monophyly of the Istiophoridae. The extant members of this family have experienced a long period of common ancestry as indicated by numerous istiophorid synapomorphies (20 transitions and 35 transversions, Fig. 8). The monophyly of *Tetrapturus*, and within this genus, clades consisting of *audax* + *albidus* and *pfluegeri* + *angustirostris* + *belone*, are also supported. The number of substitutions separating *Tetrapturus audax* from *T. albidus*, and *T. angustirostris* from *T. belone* and *T. pfluegeri* indicates that these mitochondrial lineages share a recent common ancestry (Table 3). The sequence differences between these species (<0.5%) are small compared to the maximum intraspecific sequence difference (1.8%) detected among blue marlin, *M. nigricans*, over a similar region of cytochrome *b* (Finnerty and Block, 1992). These data call into question the status of *T. audax*

and *T. albidus* as separate species as well as *T. pfluegeri*, *T. angustirostris*, and *T. belone*.

Our data conflict with other aspects of current istiophorid taxonomy at the generic level. Analysis of cytochrome *b* does not support the monophyly of the genus *Makaira*. The black marlin, *Makaira indica*, appears to be the sister group of a clade containing all other istiophorids, while the blue marlin, *M. nigricans*, is sister group of the sailfish, *I. platypterus*. The most parsimonious tree that contains a monophyletic *Makaira* is six steps longer than the shortest tree overall (158 versus 152), and on the most parsimonious tree, the *M. nigricans-I. platypterus* node is strongly supported by bootstrap analysis (85%).

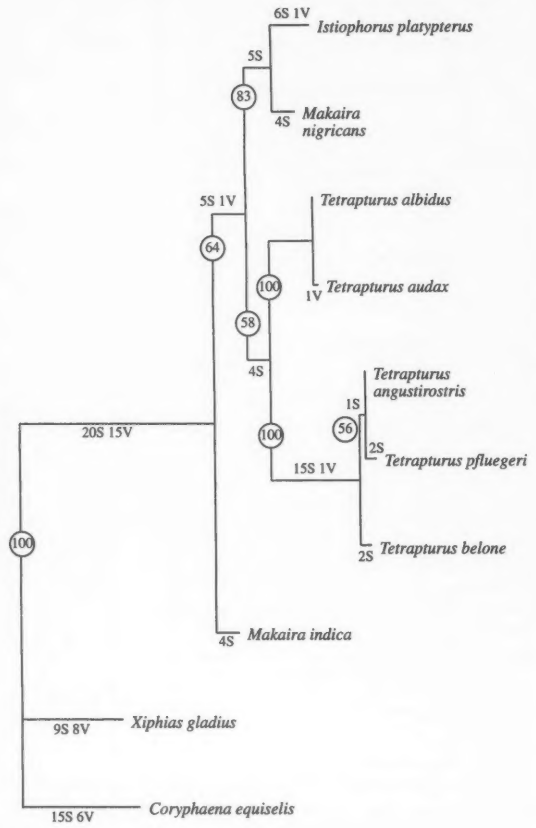

Figure 8

Phylogeny of the Istiophoridae based on 78 informative nucleotide sites. The phylogram depicted is the single most parsimonious tree identified by the exhaustive search option of PAUP 3.1 (Swofford, 1991). *Xiphias gladius* and *Coryphaena equiselis* were specified as the outgroup. Length, consistency index, and retention index are the following: L=152, CI=0.776, RI=0.721. Circled numbers at nodes indicate the percentage of trials in which a given partition between taxa is supported in 2,000 replications of the bootstrap analysis (Felsenstein, 1985). Character state transformations were inferred by using the accelerated transformation (ACCTRAN) option of PAUP: S=nucleotide transitions (A⇔G or C⇔T); V=nucleotide transversions (C/T⇔A/G). Within the Istiophoridae transitions outnumber transversions 54 to 6. Neighbor-joining (Saitou and Nei, 1987) and UPGMA dendrograms produced with Phylip 3.5 (Felsenstein, 1993) have the same topology. Distance trees were constructed by using Kimura's (1980) two parameter genetic distance, and by assuming a transition to transversion bias of 9:1.

Cytochrome *b* provides good resolution of the relationships of the genera of the tribe Thunnini (*Auxis, Euthynnus, Katsuwonus,* and *Thunnus*). According to the nucleotide data the nine Thunnini species sequenced in this study comprise two clades, one consisting of the genus *Thunnus* and one containing the other genera: *Auxis*, *Euthynnus*, and *Katsuwonus*. This distinct split in the Thunnini was proposed by Kishinouye in 1923 and is consistent with the morphological hypothesis of Collette et al. (1984). Support for the monophyly of the *Thunnus* clade is particularly robust; however, the relationships within the genus cannot be resolved without the inclusion of both *Thunnus tonggol* and *Thunnus atlanticus* which were not sequenced in this study. The number of substitutions separating *T. thynnus* from *T. maccoyii* (<0.5% sequence divergence) are small considering their status as separate species.

Discussion

Interfamilial relationships and the limits of the Scombroidei

Throughout this analysis, we have focused on the long-standing controversy over the limits of the Scombroidei and, particularly, whether billfishes are scombroids. Cytochrome *b* appears to be informative on this issue. In the two phylogenetic analyses that emphasize the more slowly evolving characters (see Figs. 4 and 7), the most parsimonious tree topology is clearly most consistent with the hypothesis that billfish are not scombroids: in each case, one or more nonscombroids share a common ancestry with the scombrid-gempylid-trichiurid clade to the exclusion of billfishes (Table 4). Therefore, according to the criterion of parsimony, the nonscombroid hypothesis is superior to the scombrid subgroup and to the scombrid sister group hypotheses. However, in our opinion, the

most parsimonious trees alone do not constitute sufficient evidence to reject these unfavored hypotheses. The question we must ask is the following: How unparsimonious are these hypotheses?

In comparing the tree topologies that support each competing hypotheses (Table 4), it is clear that our data refute the notion that billfishes share a common ancestor with the Scombridae to the exclusion of other scombroids (Gregory and Conrad, 1937; Berg, 1940; Fraser-Brunner, 1950; Collette et al., 1984; Johnson, 1986). For example, the shortest trees supporting a billfish-scombrid clade are 13% longer than the minimum-length tree based on inferred amino acid sequence (Table 4). According to the cladistic permutation test for nonmonophyly (Faith, 1991), this length difference constitutes significant evidence against the monophyly of scombrids plus billfishes. The condition that billfishes and scombrids comprise a monophyletic group is a requirement of both the scombrid subgroup and scombrid sister group hypotheses. Therefore, according to the cytochrome *b* data, we reject these two hypotheses.

The cytochrome *b* data clearly support the third hypothesis, that billfishes are not scombroids, though not as strongly as they refute the first two hypotheses. According to the inferred amino acid sequences, the shortest tree that supports scombroid monophyly places billfishes as sister group to all other scombroids and is nearly 3% longer than the most parsimonious tree overall (145 versus 141 steps). This length difference alone does not constitute significant evidence against the monophyly of the Scombroidei according to a cladistic permutation test for nonmonophyly (see Methods section; Faith, 1991). However, as previously mentioned, there are three amino acid characters that unite scombrids, gempylids, and trichiurids with *Sphyraena* to the exclusion of billfishes (amino acids 15, 16, and 169). There are no characters that unite scombrids, gempylids, and trichiurids with billfishes to the exclusion of the putative outgroups. Our study is consistent with the hypothesis that billfishes are most closely related to some percoid lineage (Nakamura, 1983; Potthoff et al., 1986). The question of which taxon is most closely related to billfishes remains unanswered. On the basis of this evidence, we support a conservative definition of the Scombroidei, including only the families Scombridae, Gempylidae,

Table 4

Comparison of three competing hypotheses of billfish (Istiophoridae and Xiphiidae) relationships based on inferred amino acid sequences from cytochrome *b*. 'na' = not applicable.

	Hypothesis		
	I: Scombrid subgroup	II: Scombrid sister group	III: Nonscombroid
Characteristics of tree which would support hypothesis	Scombridae + billfishes are a monophyletic group *Acanthocybium* is the sister group of billfishes	Billfishes are sister group of a monophyletic Scombridae	Billfishes do not compose part of a monophyletic group with any other scombroid taxon or taxa
Does the most parsimonious tree support the hypothesis?	No	No	Yes
If the answer to B is no, how much longer is the shortest tree which does support the hypothesis?	13.5% (160 steps vs. 141 steps)	13.0% (159 steps vs. 141 steps)	na
Based on the increase in tree length, can we reject the underlying hypothesis with statistical significance?	Yes (*T-PTP* = 0.01)	Yes (*T-PTP* = 0.01)	na

[1] The topology dependent permutation tail probability (*T-PTP*; Faith, 1991) was used to determine the significance of the length difference. Values of *T-PTP*≤0.05 were considered significant. See Methods section.

and Trichiuridae (Cuvier and Valenciennes, 1832; Gosline, 1968; Potthoff et al., 1986).

How can these inferences from molecular data be reconciled with the morphological data? We believe that this is an instance where molecular data complement morphological data well. Cytochrome *b* provides an unambiguous phylogenetic signal that billfishes are genetically distant from other scombroids. In contrast, the existing morphological data does not clearly discriminate between a number of hypotheses. The number of character reversals in morphological phylogenies that classify billfishes as scombroids indicates that there have been many homoplastic changes in the billfish lineage. According to the morphological evidence, either billfishes are scombroids and have undergone several reversals to the primitive state, such as their low number of vertebrae, or billfishes are not scombroids but have evolved many convergent similarities to scombroids, such as their paired lateral caudal keels.

Many of the morphological characters that unite billfishes to other scombroids, particularly to Scombridae, may be adaptations for continuous swimming, and are therefore of questionable phylogenetic value. These include hypurostegy, the projection of the caudal fin-ray bases anteriorly to cover the hypurals (Collette et al., 1984; Johnson, 1986), fusion of the hypurals (Collette et al., 1984; Johnson, 1986), and interfilamentar gill fusion (Johnson, 1986). Hypurostegy and interfilamentary gill fusion are known to have evolved convergently in nonscombroid taxa (*Luvarus imperialis* [Leis and Richards, 1984]; and *Amia calva* [Bevelander, 1934]). The molecular data presented here provide a phylogenetic signal that is independent of convergent morphological adaptations that might confound phylogenetic analysis. There has been convergent evolution in the molecular characters, but unlike many of the morphological characters mentioned, this convergent evolution does not appear to be the result of strong selection: most amino acid substitutions exchange amino acids with similar size, charge, and degree of polarity. Therefore, when compared with the existing morphological data, the phylogenetic signal in the molecular data is less likely to have been obscured by similar selective pressures acting upon distantly related lineages.

Istiophorid phylogeny

Historically, there have been numerous disagreements over the number of species within the Istiophoridae and their interrelationships (Goode, 1882; Jordan and Evermann, 1926; LaMonte and Marcy, 1941; Nakamura, 1983). This is evidenced by the synonymies for many istiophorids, e.g. the Mediterranean spearfish, *Tetrapturus belone*, has also been assigned to *Istiophorus* (Ben-Tuvia, 1953) and *Makaira* (Tortonese, 1958). The most thorough treatment of billfish systematics to date is a phenetic analysis conducted by Nakamura (1983). Nakamura recognized 11 species of istiophorid billfishes in three genera, including the designation of separate Atlantic and Indo-Pacific species for blue marlin (*Makaira nigricans* and *M. mazara*) and sailfish (*Istiophorus albicans* and *I. platypterus*).

The molecular evidence presented here agrees with Nakamura (1983) in supporting the monophyly of the genus *Tetrapturus*, and within this genus, clades consisting of *audax* + *albidus* and *pfluegeri* + *angustirostris* + *belone*. Cytochrome *b* does not support the recognition of separate Atlantic and Pacific species of blue marlin and sailfish. Previous results (Finnerty and Block, 1992) identified substantial overlap in the cytochrome *b* haplotypes found among Atlantic and Pacific populations of blue marlin. The sailfish sample in this study includes one Atlantic specimen and one Pacific specimen that differ at only two sites among 590 (0.3%). We infer from the cytochrome *b* data (Block et al., 1993; and this study) a nonmonophyletic *Makaira* and support for a clade consisting of the blue marlin (*Makaira nigricans*) and the sailfish (*Istiophorus platypterus*).

Based on the cytochrome *b* data, istiophorid taxonomy at the generic level is not concordant with phylogeny. It is premature to suggest taxonomic revision of istiophorid genera, but we believe it is imperative to obtain more molecular data, particularly from nuclear genes, to determine whether the inferences presented here can be corroborated. Furthermore, we recognize the need for an extensive cladistic analysis of istiophorid relationships based on additional morphological data. Another taxonomic issue raised by this study concerns the number of valid *Tetrapturus* species. An extensive genetic survey of several populations from each species is required to determine the number of evolutionarily independent or reproductively isolated lineages within this genus.

Relationships within the genus *Thunnus*

The systematics of the genus *Thunnus* have been well studied owing to the commercial importance of tunas and interest in physiological specializations associated with the evolution of endothermy. Collette (1978) suggested a taxonomic subdivision of the genus reflecting a split between tropical species (subgenus *Neothunnus*: blackfin tuna, *Thunnus atlanticus*, longtail tuna, *Thunnus tonggol*, and yellowfin tuna, *Thunnus albacares*) and species that inhabit cooler waters (subgenus *Thunnus*: bluefin tuna,

Thunnus thynnus, southern bluefin tuna, *Thunnus maccoyii*, albacore, *Thunnus alalunga*, and bigeye tuna, *Thunnus obesus*). According to this hypothesis, the primitive condition for the genus *Thunnus* is a tropical distribution, and the cold water tunas compose a monophyletic group united by specializations that allowed them to exploit cooler temperate or deep waters. The nucleotide analyses presented in Figures 2 and 4 are not consistent with this hypothesis. The cytochrome *b* phylogeny groups a tropical species, the yellowfin tuna, *Thunnus albacares*, with two species adapted for extremely cold water, the bluefin tuna and southern bluefin tuna (*Thunnus thynnus* and *Thunnus maccoyii*). However, it is premature to draw conclusions about relationships within the genus *Thunnus* until data are obtained from two tropical species not included in this study, *Thunnus atlanticus* and *Thunnus tonggol*.

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Assignment of *Homarus capensis* (Herbst, 1792), the Cape lobster of South Africa, to the new genus *Homarinus* (Decapoda: Nephropidae)

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Abstract.—Three species of nephropid lobsters have been recognized in the genus *Homarus*: the American and European lobsters, *H. americanus* and *H. gammarus* of the northwestern and northeastern Atlantic, respectively, and the Cape lobster of South Africa, *H. capensis*, few specimens of which have been studied until recently. Analysis of new specimens allows reconsideration of the systematic status of this species and a subsequent transfer to a monotypic new genus *Homarinus*. Far smaller than its northern relatives, with a maximum observed carapace length of 47 mm, the Cape lobster has first chelae adorned with a thick mat of plumose setae and less abundant setae on the carapace, tail fan, and abdominal pleura, whereas these setae are absent in *Homarus*. Relative length and shape of the carpus on pereopod 1, tooth pattern on cutting edges of first chelae, shape of the linguiform rostrum, large size of oviducal openings, and structure of male pleopods differ from corresponding features in *Homarus*. Comparative analysis of DNA from the mitochondrial 16s rRNA gene demonstrated considerable sequence divergence of the Cape lobster (9.7%) from its putative congeners. The magnitude of this estimate relative to that between the two North Atlantic species (1.3%) further suggests that taxonomic revision is warranted.

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Fishery Bulletin 93:97–102 (1995).

Until now, three species of nephropid lobsters have been recognized in the genus *Homarus* Weber, 1795 (see Holthuis, 1991): *H. americanus* H. Milne-Edwards, 1837, the northwestern Atlantic American lobster; *H. gammarus* (Linnaeus, 1758), the northeastern Atlantic-Mediterranean European lobster; and *H. capensis* (Herbst, 1792), the South African Cape lobster. All are found in cool or cold temperate waters, and the North Atlantic species range into subarctic waters. The northern *H. americanus* and *H. gammarus* are well-known, abundant, and economically valuable species, but the southern *H. capensis* has long been problematic because only a few specimens (13 males, 1 female) were known to exist in collections (Barnard, 1950; Wolff, 1978; Holthuis, 1991). Gilchrist (1918) had seen only three specimens and remarked (p. 46) that "it is a very rare species, and is not even known to Cape Fishermen." Kensley (1981) recorded its distribution in the Cape Province as Table Bay to East London, and recent new collections extend the range to Transkei (Kado et al., 1994).

Regardless of its rarity, sufficient specimens of the Cape lobster, living and preserved, are now available for analysis of its distribution, morphological, and genetic attributes, and systematic status. Results of our studies indicate that this species should be removed from *Homarus* and placed in a genus of its own; this paper provides supporting evidence for this action and offers supplementary descriptive information on the species.

Homarinus, new genus
Figs. 1–4

Type species—*Homarus capensis* (Herbst, 1792) by present designation and monotypy.

Description—Carapace moderately compressed, narrower than deep, sparsely setose, middorsal carina barely evident on gastric region, obsolescent on thoracic region posterior to deep cervical groove. Rostrum

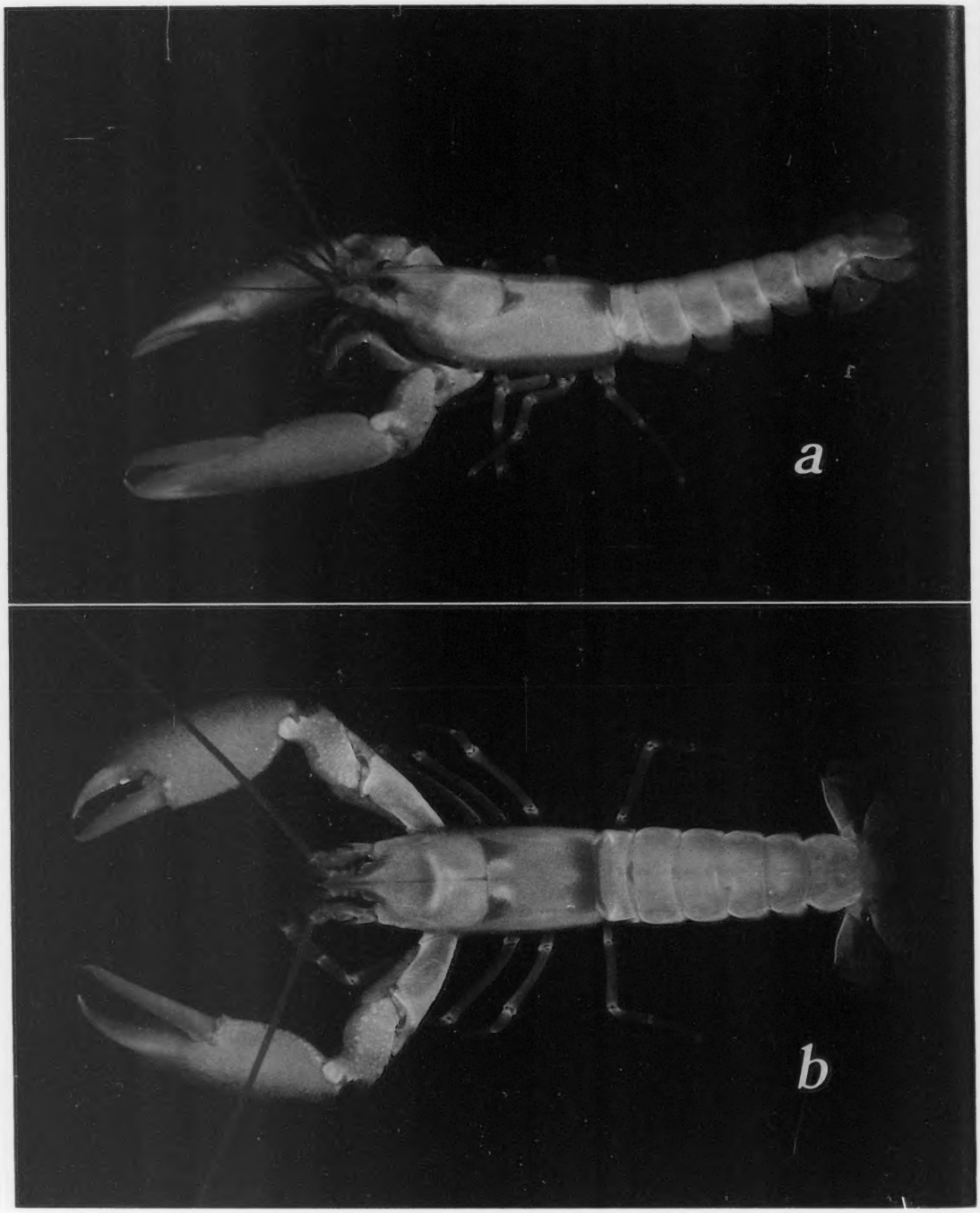

Figure 1

Homarinus capensis (Herbst). Living male, carapace length 3.41 cm, photographed in an aquarium in Sea Fisheries Research Institute, Cape Town, South Africa, by Robert Tarr. (***a***) Left lateral; (***b***) dorsal.

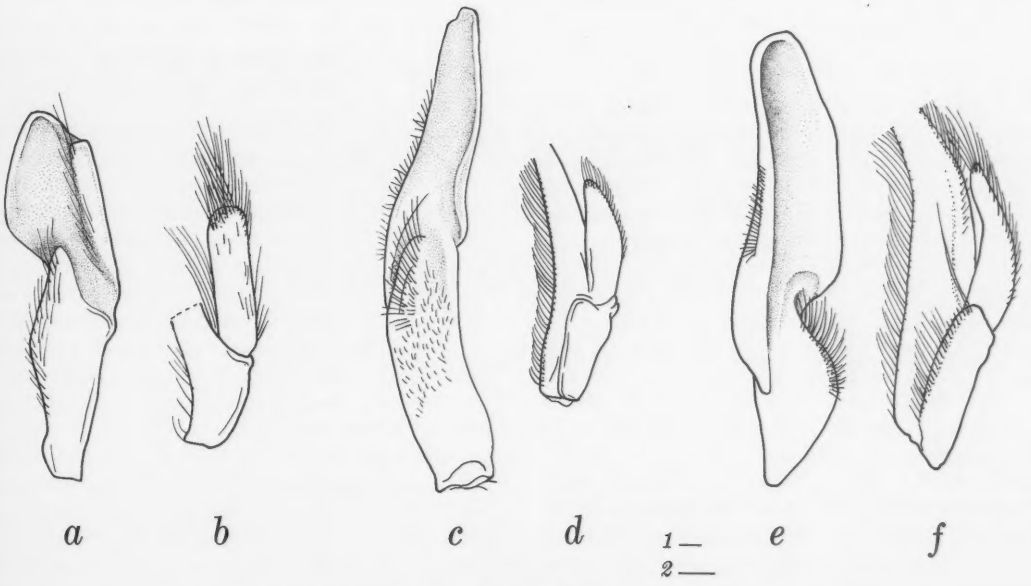

Figure 2

Male pleopods (pl); mesial views of pl 1 (slight lateral folds on tips not shown in these views), and mesial views of appendix masculina on mesial ramus of pl 2: (***a* and *b***) *Homarinus capensis*, left (USNM 251452); (***c* and *d***) *Homarus americanus*, right (USNM 13952); (***e* and *f***) *H. gammarus*, right (USNM 2085). Scale is 1 mm: bar 1 applies to *c* through *f*; bar 2 applies to *a* and *b*.

linguiform in dorsal view, broad at base where margins coalesce with orbits, margins bearing 4–6 small spines and gradually tapering anteriorly to rather abruptly pointed or narrowly rounded tip, reaching distal 1/3 of penultimate article of antennular peduncle, shallow dorsal concavity running its entire length.

Telson and uropods with thick fringe of plumose setae on distal margin and with scattered nonplumose long setae dorsally on these appendages and sixth abdominal segment. Telson as wide at base as long, with lateral margins slightly sinuous and subparallel bearing obsolescent spines and rugae, each side ending in fixed posterolateral spine; terminal margin beyond spine broadly convex; distal 1/3 of surface bearing obsolescent transverse rugae. Uropods broadly subovate, sparsely setose on dorsal surface; mesial ramus broadest near posterior margin with width about 0.73 length, row of obsolescent lateral marginal spines ending in fixed posterolateral spine; lateral ramus with width about 0.72 length, diaresis well behind midlength bearing row of fixed but irregularly worn spines ending in strongest spine at posterolateral angle.

Chelae of first pereopods with thick coat of long plumose setae on upper surface of palm, overhanging extensor margin and distributed a distance along fixed finger; similar setae on mesial surface of carpus and ventral surface of merus. Fingers not gaping; those of major chela with crushing teeth (often worn) opposed from near base to about midlength

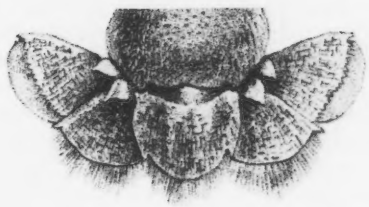

Figure 3

Homarinus capensis (Herbst), tail fan (from figure in H. Milne-Edwards, 1851).

```
Ha   ggtcgcaaacttttttgtcgatatgaactctcaaaataaataacgctgtt   50
Hg   ..................................................
Hc   ..................n...............................

Ha   atccctaaagtaacttaaatttttaatcaacaancaanggatcanttaca  100
Hg   ..................................................
Hc   ..........................ca.c.a.t........c..a.

Ha   cacnnnnnnaaatatctctgtattttaaatttaaacagttacnnaaatta  150
Hg   ........................g.....................
Hc   t..........c....t..a....a..t.....................

Ha   tatcatcgtcgccccaacgaaataattntagtatataaataatattaaac  200
Hg   ............................c.....................
Hc   ...t.........................ac..c.....g......t..

Ha   tttcaactcatctaattatatactaaattattaagctttatagggtctta  250
Hg   ...t..............................................
Hc   ..a...t..............g.a...........................

Ha   tcgtccctttaaaatatttaagccttttcacttaaaagtcaaattcaatt  300
Hg   ..................................................
Hc   ..........................................c..tg.a.

Ha   tttgtgtttgagacagtttgcttcttgtccaaccattcatacaagcctcc  350
Hg   a....a............................................
Hc   ac.t.t..c.........................................

Ha   aattaagagactaatgactatgctaccttc                     380
Hg   ............................
Hc   ......g.nn................
```

Figure 4

Partial sequence for the mitochondrial 16s rRNA gene. Sequences for *Homarus americanus* (Ha), *H. gammarus* (Hg), and *Homarinus capensis* (Hc) have been deposited with GenBank Accession Numbers U11238, U11246, and U11247 respectively. Dots indicate nucleotides identical with Ha; letters indicate nucleotide substitutions at the homologous sites. Sites marked 'n' have unresolved nucleotides.

followed by row of intermittent noncrushing moderate conical teeth with 4–6 smaller ones in intervals between them; minor chela with latter pattern of noncrushing teeth on cutting edge of each finger; tips of fingers on each chela curved toward each other and crossing.

Carpus of major chela elongate; anterior margin with two prominent spines and smaller ones between, palmar condyle subcircular and flattened, with suggestion of spines or tubercles on its anteromesial margin; dorsomesial margin strongly tuberculate and partly obscured by setae; shorter dorsolateral margin also tuberculate but less prominently so; strong low spines on mesioventral margin. Merus bearing subdistal anterolateral spine, well-separated sharp tubercles on mesiodorsal margin, and mesioventral row of fairly uniform small tubercles.

Minor chela with similar but less developed ornamentation; merus with acute spines and spiniform tubercles.

Etymology—The name *Homarinus* is derived from French *homard*, lobster, and the adjectival combining form *-inus*, resembling. The gender is masculine.

Homarinus capensis (Herbst, 1792), new combination

Synonymy—Holthuis (1986:243, fig. 1) gave an exhaustive synonymy for *Homarus capensis*, and a later (1991:59) less inclusive account. These treatments are so recent and readily available that reiteration here would be unnecessarily redundant. Succeeding reference to the species follows.

Homarus capensis.—Kado, Kittaka, Hayakawa and Pollock, 1994:72, figs. 2, 3, 4.

Material—Cape Province, South Africa. USNM 251451. 1♂, East London?, R. Melville-Smith, 92-RMS–0, Nov 1992, regurg., dismembered, carapace length (cl) 26.5 mm, short carapace length (scl) 21 mm, abdomen length (abdl) 33.0 mm. USNM 251452. 1♂, southwest Dassen Island [33°26'S, 18°05'E], regurgitated from *Sebastichthys capensis*, badly crushed and partly dismembered, R.S. Steneck, 92-D–2, 1 Dec 1992, cl 32 mm, scl 25.5 mm. USNM 251453. 1♀, Still Bay [34°23'S, 21°27'E], dismembered, R. Melville-Smith, RMS7, abdl 45 mm. USNM 251454. 1♀, Still Bay, regurg., R. Melville-Smith, RMS8, 5 mm, abdl 47 mm.

Additional specimens reported to us by R. Melville-Smith, Sea Fisheries Institute, Cape Town: 1♂, North Dassen Island, tide pool, RSS, 92-D–1, 3 Feb 1992; 1♀. Port Alfred, RMS 1; 1♂, Houghham Park, Algoa Bay; 1♂, Dassen Island, west side, RMS 3; 1♂, Cape St. Francis, RMS 4; 1♂, Cintsa Reef, East London, RMS 5; 1♂, Sunday's River mouth, RMS 6; 2♂, Cape St. Francis, RMS 9 and 10; 1♀, Haga Haga, Transkei coast, RMS 11.

Description—As for genus with addition of the following details.

Abdominal pleura well developed, with rounded angles; pleuron of segment 1 small; pleuron of segment 2 broad, overlapping first and third pleura; pleura 3–4–5 with anteroventral angle rounded, posterolateral angle subrectangular; pleuron of segment 6 rounded ventrally, posterolateral angle rounded and confluent with anterolateral angle of telson.

Telson with dorsal setae distributed in 3 longitudinal tracts, central and submarginal on either side; central tuft proximally in midline and another near each anterolateral corner; sparse similar setae on abdominal pleura; lateral ramus of uropod with ventral submarginal row of setae laterally.

Eyes with distal edge of cornea slightly exceeding level of basicerite tip; this tip reaching to midlength of narrowly rounded antennal scale exceeded by its very strong anterolateral spine (rarely doubled) reaching distal edge of penultimate article in antennular peduncle; latter falling short of distal margin of terminal article in antennal peduncle.

Epistome with median anterior spine closely flanked at either side by shorter rounded spine.

Cheliped of pereopod 1 having fixed finger with narrowed extensor margin set off by shallow submarginal groove. Palm with compound row of low forward pointing spines and tubercles on flexor surface, similar development on extensor edge originating at carpal condyle and running along proximal margin of palm, across its basal end, and distally for a distance along palm.

Oviducal opening on coxa of pereopod 3 oval; its axes 1.3 × 1.8 mm on measured female noted below.

Pleopod 1 with distal article broader than shaft and hollowed mesially, forming flattened tubular opening when appressed to opposite member, tip irregularly rounded. Pleopod 2 with appendix masculina on mesial aspect of endopod bearing tuft of strong setae at apex.

Uropods with protopodite bearing 2 strong spines overhanging proximal end of mesial and lateral ramus respectively.

Variation—There is minor variation in development of spines, tubercles, etc., among the two females and two males examined. According to Stebbing (1900), sides of the rostrum may have 5, 6, or 7 spines on the margin. Density of setae on exoskeletal parts is subject to considerable variation, owing perhaps to recency of molting, age, or abrasion after preservation.

Color—Color of a living animal is shown in Figure 1. Published records summarized by Holthuis (1986) indicate that color may depart considerably from that shown here: coral-red to tawny or reddish yellow, which may have resulted from postmortem changes; or, in the fresh state, "of a rather dark olive colour, not dissimilar to that of the Northern lobster" Gilchrist (1918:45).

Molecular characterization—Comparative analysis of a portion of the 16s ribosomal RNA gene from mitochondrial DNA (mtDNA) was conducted by using standard protocols (Kocher et al., 1989). Mitochondrial DNA's purified by CsCl ultracentrifugation (Lansman et al., 1981) were amplified by PCR with the conserved primers 16sar and 16sbr of Palumbi et al. (1991). Following asymmetric amplification (*Homarus americanus* and *H. gammarus*) or cycle-sequencing (*Homarinus capensis*), DNA's were manually sequenced by the dideoxy chain-termination method of Sanger et al. (1977). Aligned sequences are presented in Figure 4. Sequence divergence between taxa was estimated by using the two-parameter method of Kimura (1980). Sequence divergence between *Homarus americanus* and *H. gammarus* was 1.3%, whereas average divergence between these two species and *Homarinus capensis* was 9.7%. The 16s rRNA gene is one of the most slowly evolving regions of the mtDNA molecule (Xiong and Kocher, 1994); this conservative property makes it particularly useful for comparative studies among distantly related taxa. Though there is no formal recognition of equivalence between levels of sequence divergence and taxonomic rank (Hillis and Moritz, 1990), it is clear that the relative magnitude of divergence can be a useful taxonomic indicator (Avise, 1994). The magnitude of sequence differentiation that we observed between *H. capensis* and the two North Atlantic taxa strongly suggested the existence of two discrete clades. Molecular divergence reinforced our conclusions from the reexamination of the morphology of these species.

Remarks—Morphological differences between *Homarinus capensis* and the two species of *Homarus* are clear cut. Perhaps the most obvious differences are that *Homarinus capensis* has a dense coat of setae on the outer surface of the palms and on other articles of the chelipeds (P1), and scattered setae distributed over the carapace, tail fan, sixth abdominal segment, and pleurae of the remaining abdominal segments; *Homarus americanus* and *H. gammarus* are smooth and glabrous. The telson of *Homarinus* has subparallel sides and its exposed surface bears many obsolescent transverse rugae (Fig. 3); the telson of *Homarus* species has sides converging toward the tip, giving a subtriangular shape. First pleopods are more elongate and slender in *Homarus* species than in *Homarinus* (Fig. 2).

The two species of *Homarus* attain large size (Wolff, 1978), whereas *Homarinus capensis* appears to be much smaller at maturity. No ovigerous females of *H. capensis* have been found, but openings of the oviducts are at least twice the size of those on comparably sized specimens of the species of *Homarus* (see Kado et al., 1994). This suggests that there are fewer eggs with accelerated larval development in *Homarinus capensis* relative to slower larval devel-

opment from smaller more numerous eggs in *Homarus* species (Kado et al., 1994).

Acknowledgments

Our conclusions converged independently from two viewpoints. A. B. W. and other carcinologists have long understood grounds for generic separation of the Cape lobster from *Homarus* on the basis of morphology. I. K. and R. S. S. concluded this on the basis of genetic divergence and were well into their analysis before forces were joined. A. B. W. drafted the systematic section and assembled the jointly produced text. Keiko Hiratsuka Moore rendered drawings of the pleopods. G. C. Steyskal provided advice on the choice of a new generic name. The manuscript was critically reviewed by W. Glanz, R. B. Manning, and T. A. Munroe. We are indebted especially to colleagues at the Sea Fisheries Institute, Cape Town, South Africa, who helped us in this study; Roy Melville-Smith provided materials and information, and Robert Tarr photographed the living specimen of Cape lobster. George M. Branch, University of Cape Town, provided logistic support and aided in specimen acquisition. Yan Kit Tam and Alex Parker provided sequence data. R. S. S. was supported by grants from the South African Foundation of Research Development, the Visiting Scholar Fund, and the Student Fund for Visiting Scholars of the University of Cape Town. Molecular work was supported by NOAA Sea Grant (NA90AAD-SG499) and NSF (EHR-9108766 and OCE-9203342).

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Ageing of three species of tropical snapper (Lutjanidae) from the Gulf of Carpentaria, Australia, using radiometry and otolith ring counts

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Abstract.—A radiometric ageing method was used to resolve conflicting results from ageing tropical lutjanids based on annual ring counts in whole and sectioned otoliths. The number of rings detected in sectioned otoliths of *Lutjanus erythropterus*, *L. malabaricus*, and *L. sebae* from unexploited populations in the Gulf of Carpentaria, Australia, were 1.6 to 2.4 times the number found in whole otoliths. To obtain an independent estimate of age, we measured $^{210}Pb/^{226}Ra$ radioactive disequilibria of both whole and cored otoliths. As all species had high levels of ^{226}Ra, they could be aged with relative accuracy by this method. Samples of whole otoliths and cores with a similar ring count had similar radiometric ages. In samples whose sectioned and whole-otolith ages differed by more than 4 years, the whole otolith ring count agreed better with the radiometric age (for an uptake activity ratio R=0.0). This result stands in marked contrast to the radiometric age validation of section counts for slow-growing, long-lived fish inhabiting temperate to subtemperate waters. In this region, all species lived less than 10 years and grew to a maximum size of up to 600 mm SL. They reached a similar length in one year, but *L. erythropterus* grew faster than the other two species thereafter. The sexes had the same growth rates. Our results were similar to those found for these species elsewhere and suggest that in tropical fishes, such as lutjanids, rings observed in sectioned otoliths and other hard parts may not be formed annually. Where possible, ages derived from counts in these structures should be verified by independent methods.

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Tropical fishes can be difficult to age because many species do not deposit annual rings in their hard parts (Longhurst and Pauly, 1987). Lutjanids, which are highly valued commercial fishes in the tropical Indo-Pacific region, often have ring patterns in their hard parts that are difficult to interprete (e.g. Davis and West, 1992). The age and growth of many lutjanid species have been well studied and the results of these studies have formed the basis of age-structured stock assessments upon which the management of these fisheries is based (e.g. Sainsbury, 1988).

In the western Pacific, *Lutjanus malabaricus* has been the most widely studied lutjanid, as it is the main catch of trawl and line fisheries in northern Australia, adjacent Indonesian waters, and in the South China Sea. The reported maximum age (up to 10 yr) and growth parameters differ both between regions (Lai and Lui, 1974, 1979) and within one area (northern Australia: Lai and Lui, 1979; Chen et al., 1984; Edwards, 1985; McPherson and Squire, 1992). These studies estimated age from growth rings in vertebrae (Lai and Lui, 1979; Chen et al., 1984; Edwards, 1985) or in whole otoliths (McPherson and Squire, 1992). The latter method may underestimate the age of longer-lived species because of the difficulty of distinguishing all the growth rings (Casselman, 1974).

The timing of formation of annual growth rings in *Lutjanus* from northern Australia has not been fully verified. Several authors (Lai and Lui, 1974, 1979; Chen et al., 1984; Yeh et al., 1986; Davis and West, 1992) have concluded that the outer ring is probably deposited

annually, but the season when this ring is formed varies between studies and species. Such ambiguities cast doubt on the validity of the conclusions and suggest that differences in the estimated growth rate and age of *Lutjanus* populations may be related to problems of interpretation rather than to biological differences.

A radiometric method has recently been used successfully to estimate the age of long-lived fishes (Bennett et al., 1982; Fenton et al., 1991). This method uses the known decay rates of isotopes of Radium–226 (^{226}Ra) and Lead–210 (^{210}Pb) in bony parts to estimate the age of fish. It does not rely on operator interpretation to estimate age and therefore is particularly useful for ageing long-lived species where the growth rings are often not clearly defined (Casselman, 1974).

The objectives of the present study were 1) to estimate the age and growth of *Lutjanus malabaricus*, *L. erythropterus*, and *L. sebae* from the Gulf of Carpentaria by counting rings in whole and sectioned otoliths; and 2) to use the ^{210}Pb/^{226}Ra radiometric ageing method to make an independent age estimation of the same fish.

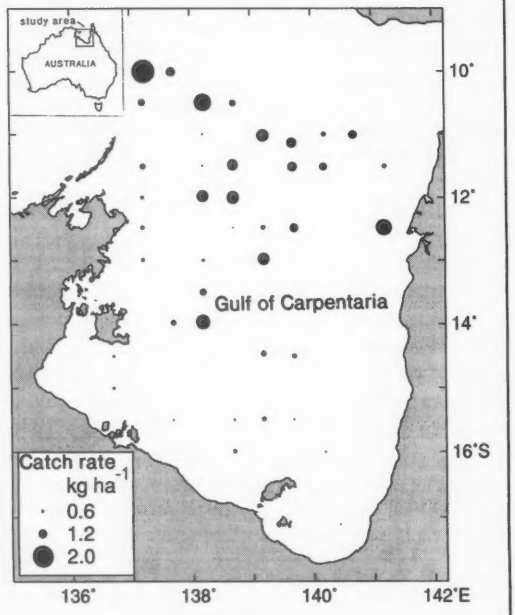

Figure 1

Map of the Gulf of Carpentaria showing the distribution and relative abundance of *Lutjanus erythropterus*, *L. malabaricus*, and *L. sebae* during a systematic survey in November 1990.

Materials and methods

Sampling

Most samples of *Lutjanus erythropterus*, *L. malabaricus*, and *L. sebae* were collected during a systematic survey of the Gulf of Carpentaria between long. 136° and 142°E in November 1990. Two similar random-sampling surveys were made across the northern Gulf of Carpentaria (north of lat. 14°30'S) in November 1991 and January 1993. Samples of *Lutjanus malabaricus* were also collected during a survey of eight areas in the Gulf of Carpen-taria by the commercial trawler *Clipper Bird* in June 1990 (Fig. 1). Details of survey design, trawl gears, and trawl durations are given in Blaber et al.(1994).

Commercial-sized *Lutjanus malabaricus* (1–3 kg) were obtained from fish retained for sale after the June 1990 survey. During the systematic survey in November 1990, all specimens of the three target species of lutjanids were retained for ageing studies. In November 1991 and January 1993, only fish from length classes underrepresented in previous samples were processed. All fish were measured (standard length [SL] in mm), weighed (±1 g), and sexed, and both sagittae were removed, dried, and stored in labelled bags for future analysis.

Radiometry

Radioanalysis requires about 1 g of sample material; therefore, fish were pooled to obtain the necessary sample weight. For juveniles, up to four otoliths were required to obtain this weight. Otoliths used for radioanalysis were chosen in two ways. First, for each species, otoliths from juvenile, maturing, and mature fish that had the same sectioned-otolith ages, similar otolith weights, and similar sizes, and came from the same region of the Gulf of Carpentaria were pooled for radioanalysis. Second, otoliths of the same whole-otolith age and from fish of similar size were abraded with a mechanical sander to a central core approximating the weight (see Table 2), length, and shape of the otolith of a fish whose whole-otolith age was 3 (otolith length=11.6 ± 0.7 for *L. erythropterus*; 12.7 ± 0.4 for *L. malabaricus*; and 11.4 ± 0.4 for *L. sebae*). The exception was sample 2490 for which otoliths were ground to a core age of 2 (weight 0.156 ± 0.005 g; otolith length 9.2 ± 0.16 mm; n=92). The otolith nucleus at the center of the cores was located by examining intact otolith morphology and by sectioning other samples (Campana et al., 1990). This age is less than the age at sexual maturity for all species.

The method of radioanalysis of the otoliths is detailed in Fenton et al. (1990, 1991). It involves measuring the specific activity of ^{226}Ra:^{210}Pb by alpha-spectrometry. Because of the extremely small specific activities measured (0.01–0.1 dpm·g^{-1} for Polonium–210 [^{210}Po]), cleanliness is of the utmost importance in the analytical procedure. Every item of laboratory ware that contacted the otolith solutions and otoliths was chemically decontaminated in alkaline 0.05M Na_4EDTA (pH 10.5). The otoliths were washed and rinsed several times in this solution, then washed several times in 0.1M HCl (<10 s) and finally washed twice in water.

Our analyses of ^{210}Pb, via its short-lived daughter-proxy ^{210}Po, and ^{226}Ra were made with high-resolution alpha-spectrometers according to the methods of Fenton et al. (1990). The mean ^{210}Po reagent blank was 0.0071 ± 0.0012 dpm. Recovery of ^{210}Po was always at least 90% and instrument background counts (for ^{208}Po and ^{210}Po) were less than one count·d^{-1}. ^{226}Ra was analyzed by a direct alpha-spectrometry method and chemical yield was measured by gamma spectrometry of a Barium–133 (^{133}Ba) tracer (Fenton et al., 1990, 1991). Mean activity of the ^{226}Ra blanks was 0.0174 ± 0.0026 dpm, which was lower than in previous studies (e.g. Bennett et al., 1982; Fenton et al., 1991) owing to careful control of reagents. Recovery of ^{226}Ra (as estimated by the recovery of ^{133}Ba tracer) was greater than 85% for all samples.

Data analysis

The ages of whole otoliths were calculated on the basis of a single constant (linear) growth rate by the equation originally derived by Bennett et al. (1982):

$$A = 1-(1-R)\frac{1-e^{-\lambda t}}{\lambda t}, \qquad (1)$$

where A=the ratio of the activity of ^{210}Pb to ^{226}Ra activity at time t ($^{210}Pb/^{226}Ra)_t$; R=ratio of ^{210}Pb to ^{226}Ra at the time of deposition [$(^{210}Pb/^{226}Ra)_0$]; and λ=decay constant for ^{210}Pb (0.03114 yr^{-1}). Assumption of a single linear mass growth rate produces radiometric ages that are greater than those that would result from assumption of an exponential (non-linear) rate, the bias always favoring a higher value (Campana et al., 1993). It should be understood that using this assumption (linear mass growth of the otolith) will produce age estimates that always overestimate the real age.

For otolith cores, ages were calculated from Smith et al.'s (1991) equation:

$$A = \left(1-e^{-\lambda(t-T)}\right)+\left(1-(1-R)\frac{\left(1-e^{-\lambda T}\right)}{\lambda T}\right)e^{-\lambda(t-T)}, \qquad (2)$$

where all parameters are the same as in the previous model, except T, which is the estimated age of the otolith core. A linear mass growth model was assumed only up to the age of the core. The initial uptake $^{210}Pb/^{226}Ra$ activity ratio was generally assumed to be R=0.0. This is the most conservative value, and so radiometric age estimates derived with this value must overestimate the maximum possible age of the sample. The above equations were solved numerically by a Newton-Raphson iteration method (Fenton et al., 1991).

Stable element analysis

The levels of lead and barium in otoliths are presumed to act as stable equivalents of ^{210}Pb and ^{226}Ra and so can be used to assess the uptake of the radioactive isotopes and to normalize the radiometric data (Fenton and Short, 1992). Therefore, the concentrations of stable lead, barium, strontium (Sr), and calcium (Ca) in each otolith sample were measured for an aliquot of each dissolved otolith solution used in the radiometric analysis. Each solution was analyzed by inductively coupled plasma mass spectrometry for lead and barium and by inductively coupled plasma atomic emission spectrometry for strontium and calcium.

Otolith ageing

Pairs of otoliths from each fish were cleaned of excess tissue, dried at 60°C for 24 h, weighed (± 0.1 mg) and measured along the longitudinal axis with dial calipers (± 0.05 mm). One otolith of each pair was embedded in polyester resin and cross-sectioned with a diamond saw (Augustine and Kenchington, 1987). Thin sections (approximately 200 µm) of each otolith were bonded to microscope slides with thermoplastic cement. Each section was polished on both faces with 800-grit wet-and-dry carborundum paper before being examined with a video-enhanced light microscope attached to a microcomputer with precise distance-measuring software. The rings (presumed annuli) were counted and the distance between them measured along the dorsal axis adjacent to the sulcus, where they were most clearly distinguishable. In whole otoliths, the rings were counted against a strong background point light source.

Counts of rings in all whole and sectioned otoliths were made independently by two readers. When the ring counts differed, the otoliths were reexamined by both readers. If the counts still differed by more

than one, the data were discarded (<5% of all otoliths). If counts differed by one, the higher value was chosen (10% of otoliths). The relative frequency of these discrepancies was similar for all species.

Data analysis

The length-at-age data were fitted to the reparameterized von Bertalanffy growth curve of Francis (1988). This method has the advantage that the parameters estimated are independent and can be compared directly between species and populations.

Most previous studies of lutjanid age and growth have fitted the von Bertalanffy growth equation to data on length at age (e.g. Lai and Lui, 1979; Manooch, 1987; Davis and West, 1992). However, the estimated parameters L_∞, K, and t_0 either do not have direct biological meaning (e.g. Knight, 1968; Schnute and Fournier, 1980; Ratkowsky, 1986) or are extrapolations from the data (Ratkowsky, 1986). Francis (1988) extended the equation of Schnute and Fournier (1980) to derive a new set of parameters L_1, L_2, and L_3 (his l_ϕ, l_χ, and l_w), which correspond to the length at the lower, middle, and upper limits of any arbitrarily defined age range, such that:

$$L_t = L_1 + (L_3 - L_1)\left(1 - r^{2(t-\phi)/(w-\phi)}\right)\Big/(1 - r^2), \quad (3)$$

where $r = (L_3 - L_2)/(L_2 - L_1)$; L_t is the mean length of a fish at age t; and L_1, L_2 and L_3 are the length at the lower, middle, and upper limits of two arbitrary ages ϕ and w. By fitting a curve of this form, extrapolations beyond the data are avoided, as the three fitted parameters are chosen from within the range of the data and hence can be directly compared with the results of previous studies.

In this study, we set $\phi = 1$ ring and $w = 6$ rings for each species. This equation has the advantage that the age range to be examined can be chosen by the investigator, rather than having to be the largest and smallest age classes found, as required by the Schnute and Fournier (1980) equation. These parameters (L_1, L_2, and L_3) can also be expected to have similar properties to those of Schnute and Fournier (1980) and not to show the high negative correlation between L_∞ and K (Francis, 1988).

All parameters were estimated by an iterative least-squares method (SAS NLIN procedure with the Marquardt option; SAS, 1989). Vaughan and Kanciruk (1982) found that this procedure consistently showed the least bias in parameter estimates, converged rapidly, and provided more precise estimates than did standard linear techniques. A measure of goodness-of-fit was obtained by calculating an r^2 value from the residual and the explained sums of squares derived from the least-squares regression.

Relationship of ring counts in whole and sectioned otoliths with radiometric ages

The estimated age from ring counts in whole and sectioned otoliths used in the radiometry were compared for all species by two methods. First, the relationship between radiometric age and whole and sectioned otolith ages of the same fish were plotted. If the slope of the relationship was not significantly different from 1, the results of the two methods were considered to be in close agreement. Second, the two ageing methods were compared with the radiometric ages with a Wilcoxon matched-pairs ranks test (Conover, 1980). The two hypotheses tested were 1) that whole otolith ring counts underestimated true age (radiometric age) or 2) that sectioned otolith ring counts overestimated true age.

Results

Radiometry

Lutjanus erythropterus—The specific activity of ^{226}Ra and the ^{210}Pb/^{226}Ra activity ratio differed among the three samples of *L. erythropterus* (Tables 1 and 2). The activity ratio was highest in the cored sample (0.118 ± 0.031; Table 2). Ring counts in whole otoliths were linearly related to otolith mass (Fig. 2A) and ring count in sectioned otoliths, though the relationship was significantly weaker ($P<0.05$). Radiometric age estimates were calculated on the basis of a single constant (linear) growth rate for otolith mass, which removes the need to include the mass growth relation in the radiometric age calculation (Eq. 1). Under the assumption of a constant growth model, radiometric age estimates were most similar to those obtained from the ring counts in whole otoliths (Table 2). The match was best for the cored otolith sample where model assumptions are less stringent (sample 2673).

Lutjanus malabaricus—The specific activity of ^{226}Ra in *L. malabaricus* otoliths differed among samples and among size classes (Tables 1 and 2). The ^{210}Pb/^{226}Ra activity ratios ranged from less than 0.027 to 0.212 and varied to a similar degree in cored and whole-otolith samples (Table 2). Otolith weight was linearly related to the number of rings in whole otoliths (Fig. 2B), and this relationship was stronger than that for counts from sectioned otoliths

Table 1

Elemental composition of otoliths of three species of *Lutjanus* used in the radiometric analysis. Whole = whole otoliths used; cored = otoliths cored to age 3+ (*L. erythropterus*) or 2+(*L. malabaricus* and *L. sebae*). Numbers in parentheses represent repeated analyses of a sample in which several otoliths of similar whole and sectioned age had been combined.

Species	Sample	Whole/or cored	^{226}Radium dpm·g^{-1} (± 1σ)	^{210}Lead dpm·g^{-1} (± 1σ)	Lead (Pb) (ppm)	Barium (Ba) (ppm)	Pb/Ba mass ratio	Strontium (Sr) (ppm)	Calcium (Ca) (ppm)	Sr/Ca mass ratio
L. erythropterus	2065	Whole	0.2277 ± 0.0132	0.0174 ± 0.0047	0.08	18.7	0.004	2,800	395,000	0.0071
	2066	Whole	0.1623 ± 0.0087	0.0070 ± 0.0035	0.19	11.5	0.016	2,720	398,000	0.0068
	2673	Cored	0.1331 ± 0.0087	0.0157 ± 0.0040	0.66	9.2	0.071	—	—	—
L. malabaricus	2062(2)	Whole	0.2390 ± 0.0111	−0.0025 ± 0.0045	0.27	8.2	0.033	2,915	462,000	0.0063
	2063	Whole	0.0728 ± 0.0066	0.0067 ± 0.0042	3.49	6.5	0.537	3,330	470,000	0.0071
	2063(2)	Whole	0.0582 ± 0.0049	−0.0010 ± 0.0025	0.22	13.4	0.016	1,990	321,000	0.0062
	2063(3)	Whole	0.0916 ± 0.0053	0.0072 ± 0.0017	0.55	4.8	0.114	2,240	399,000	0.0056
	2064	Whole	0.2118 ± 0.0164	0.0173 ± 0.0024	2.28	5.8	0.390	3,000	395,000	0.0076
	2438	Whole	0.1014 ± 0.0064	0.0068 ± 0.0036	0.41	5.3	0.080	2,005	426,000	0.0047
	2439	Whole	0.2942 ± 0.0141	0.0133 ± 0.0043	0.06	7.5	0.008	2,250	415,000	0.0054
	2440	Whole	0.1080 ± 0.0068	0.0135 ± 0.0029	0.18	6.2	0.029	2,910	438,000	0.0067
	2489	Cored	0.1678 ± 0.0088	0.0356 ± 0.0055	<0.09	6.7	<0.014	2,160	407,000	0.0053
	2490	Cored	0.1219 ± 0.0078	0.0180 ± 0.0037	<0.09	6.2	<0.014	2,040	416,000	0.0049
L. sebae	2068	Whole	0.1036 ± 0.0064	0.0139 ± 0.0034	3.13	8.7	0.360	2,360	396,000	0.0060
	2069	Whole	0.1046 ± 0.0058	0.0312 ± 0.0037	1.38	8.1	0.170	2,510	398,000	0.0063
	2070	Whole	0.0460 ± 0.0042	0.0100 ± 0.0023	0.46	5.0	0.092	—	—	—
	2647	Cored	0.2143 ± 0.0114	0.0373 ± 0.0049	0.50	11.5	0.043	—	—	—
	2648	Cored	0.1756 ± 0.0099	0.0458 ± 0.0052	0.66	9.4	0.071	—	—	—

Table 2

Results of radiometric and direct ageing otoliths of *Lutjanus malabaricus, L. erythropterus* and *L. sebae* from the Gulf of Carpentaria. Radiometric ages were calculated by using a constant growth rate model and by using R = 0.0 (where R = initial ^{210}Pb:^{226}Ra activity ratio at time of deposition). All errors in radiometric age estimates expressed at 1σ level (n = number of otoliths in sample). SE = Standard error. Numbers in parentheses represent repeated analyses of a sample in which several otoliths of similar whole and sectioned age had been combined.

Species	Sample	n	Mean length (mm) ± SE	Mean otolith mass (g) ± SE	Whole otolith age	Sectioned otolith age	^{210}Pb:^{226}Ra activity ratio	Radiometric age
L. erythropterus	2065	3	316 ± 2	0.3315 ± 0.0186	3	3	0.076 ± 0.021	5.1 ± 1.5
	2066	2	364 ± –	0.3875 ± –	3.3	6	0.043 ± 0.022	2.8 ± 1.5
	2673	3	368 ± 7	0.4750 ± 0.0507	4	9	0.118 ± 0.031	5.5 ± 1.1
L. malabaricus	2062(2)	2	310 ± –	0.4852 ± –	3	3	<0.027 (95% CL)	<1.8 (95% CL)
	2063	2	350 ± –	0.5775 ± –	4	6	0.092 ± 0.058	5.7 +4.3,–4.0
	2063(2)	2	348 ± –	0.6170 ± –	4	6	<0.069 (95% CL)	<4.6 (95% CL)
	2063(3)	3	346 ± 7	0.6118 ± 0.0133	4	6	0.079 ± 0.019	0.8 ± 0.8
	2064	3	443 ± 7	1.5591 ± 0.0493	6.7	14	0.082 ± 0.013	5.6 ± 0.9
	2438	4	250 ± 2	0.2517 ± 0.0063	3	3.5	0.067 ± 0.036	4.5 +2.6,–2.5
	2439	1	650	2.1155	9	13	0.045 ± 0.015	3.0 ± 1.0
	2440	1	560	2.1111	7	19	0.125 ± 0.028	8.8 + 2.2,–2.1
	2489	4	455 ± 10	1.6185 ± 0.1349	9	13	0.212 ± 0.035	8.7 +1.5,–1.4
	2490	5	422 ± 7	1.1055 ± 0.0501	8	8	0.148 ± 0.032	6.1 ± 1.2
L. sebae	2068	4	222 ± 5	0.2429 ± 0.0261	2	3	0.134 ± 0.034	9.5 + 2.7,–2.6
	2069	2	304 ± –	0.6152 ± –	3.5	7	0.298 ± 0.039	24.2 +4.2,–3.9
	2070	2	399 ± –	1.5658 ± –	5.5	15	0.217 ± 0.054	16.4 +5.1,–4.6
	2647	3	400 ± 15	1.4462 ± 0.0184	5	12.3	0.174 ± 0.023	7.1 ± 1.0
	2648	2	462 ± –	2.1000 ± –	7	15.5	0.261 ± 0.033	11.2 +1.5,–1.4

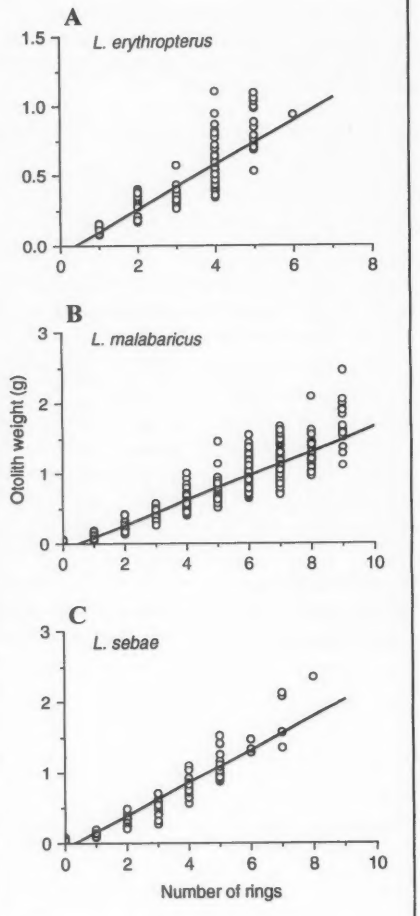

Figure 2

The relationship between otolith weight and the number of rings counted in whole otoliths of (**A**) *L. erythropterus*, (**B**) *L. malabaricus*, and (**C**) *L. sebae*.

($P<0.05$). Under the assumption of a constant mass growth model, radiometric age estimates were again most similar to those found for whole otolith ring counts. The match was best for samples of cored otoliths (2489, 2490) where assumption of a mass growth model is almost absent (Table 2).

Lutjanus sebae —The specific activity of ^{226}Ra and the $^{210}Pb/^{226}Ra$ activity ratio varied less between samples in *L. sebae* than in the other species (Tables 1 and 2). As with the other species, otolith weight was linearly related to the ring counts of whole otoliths; therefore, a single constant growth rate was assumed in interpretation of the radiometric data (Fig. 2C). The radiometric age estimates of intact otolith samples of juveniles (2068, 2069, 2070), based on the assumption of no allogenic ^{210}Pb uptake in the otoliths (R=0.0), were higher than the ring counts for both sectioned and whole otoliths (Table 2). Samples 2068 and 2069 were probably subject to high rates of allogenic ^{210}Pb uptake, as indicated by the high stable Pb/Ba mass ratios (Table 1). Radiometric ages of both sets of cored otoliths were most similar to the age estimates based on whole otolith counts. However, both these samples (2647 and 2648) had very low stable Pb/Ba mass ratios (Table 1). Modelled radiometric ages of *L. sebae* samples (both whole and cored) for different values of R indicate that $R = 0.10$ best matches the ring count of whole otoliths (Table 3).

Lead:Barium ratios

The stable lead:barium ratios of all samples were plotted against radiometric age assuming an initial activity ratio $R = 0.0$ (Fig. 3). Neither *L. malabaricus* nor *L. erythropterus* showed an increase in the ratio with increasing age. However, in four of the five *L. sebae* samples radiometric age increased rapidly with increasing stable lead (Fig. 3).

Otolith ageing

Lutjanus erythropterus—The growth curves of *L. erythropterus* based on ring counts in whole otoliths

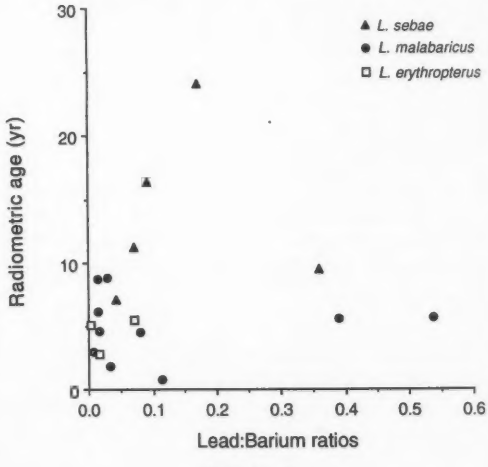

Figure 3

Plot of the relationship between radiometric age and lead:barium ratios for the three species of *Lutjanus*.

Table 3

Radiometric age estimates of samples of both whole and cored(*) *Lutjanus sebae* otoliths for a range of initial uptake $^{210}Pb/^{226}Ra$ activity ratios (R). [ ] denotes best match of radiometric age to whole age. Standard errors of samples 2069 and 2070 were unavoidably large, resulting in a poor agreement with either whole or sectioned ages. For whole otoliths, radiometric age estimates assumed linear mass growth for otoliths throughout. For cored otoliths, radiometric age estimates assumed linear mass growth for otoliths only to age 2+, thereafter they were irrelevant.

Sample number	Mean whole otolith age	Mean sectioned otolith age	Radiometric age (yr) for various R values			
			$R_{0.0}$	$R_{0.05}$	$R_{0.1}$	$R_{0.15}$
2068	2	3	9.5+2.7,–2.6	6.0+2.7,–2.5	[2.5+2.5,–1.9]	0.3±0.4,–0.3
2069	3.5	7	24.2+4.2,–3.9	20.4+4.1,–3.8	16.6+4.0,–3.7	12.5+4.1,–3.5
2070	5.5	15	16.4+5.1,–4.6	12.8+4.9,–4.5	9.1+4.9,–4.4	[5.4+4.7,–3.8]
2647*	5	12.3	7.1±1.0	6.0±0.9	[4.3±0.9]	1.9±0.9
2648*	7	15.5	11.2+1.5,–1.4	9.6+1.5,–1.4	[7.8+1.5,–1.4]	5.5±1.4

differed from those obtained from sectioned otoliths (Figs. 4A and 5). Fewer rings were counted in whole than in sectioned otoliths but they were linearly related (Fig. 6A; whole otolith count=0.40 × (sectioned otolith count) + 1.02; r^2=0.69, $F_{1,170}$=368.7, P<0.0001). However, the length-at-age data overlapped for all the size classes detected in whole otoliths (Fig. 4A). Both sexes had similar growth parameters based on whole otlith ring counts (P>0.3) (Table 4) and lived to a similar age.

***Lutjanus malabaricus*—**The growth curves expressing the best fit of length-at-age data from both sectioned otoliths and whole otoliths show significant differences (P<0.05) in the estimated growth rates (Fig. 4B). More rings were counted in sectioned otoliths than in whole otoliths from the same fish (Fig. 6B), but were linearly related (whole otolith count=0.64 × (sectioned otolith count) + 0.79; r^2=0.81, $F_{1,869}$=3614.7, P<0.001).

Growth parameters of the reparameterized von Bertalanffy equation of male and female *L. malabaricus* did not differ except for L_3; this parameter was larger in males (P<0.05). Not all fish collected were sexed, but the growth parameters of the combined equation differed from that obtained from the subsets that were sexed (Table 4).

***Lutjanus sebae*—**Ages based on counts of sectioned and whole otoliths differed significantly in *L. sebae* over 350 mm SL (P<0.05; Fig. 4C). More rings were detected in the otoliths of these fish when they were sectioned than when examined intact, although the number of rings detected by the two methods were linearly related (Fig. 6C; whole otolith count=0.50 × (sectioned otolith count) + 0.19; r^2=0.80, $F_{1,140}$=546.0, P<0.0001).

The growth parameters of the reparameterized von Bertalanffy equation were similar for both sexes (Table 4). *Lutjanus sebae* were larger at one year (L_1)

Table 4

Growth parameters (SL ± SE) of the reparameterized von Bertalanffy growth equations for *Lutjanus malabaricus, L. erythropterus,* and *L. sebae* (1–6 rings) from the Gulf of Carpentaria (r^2 = nonlinear estimate of goodness-of-fit).

Species	Sex	n	L_1 ± SE	L_2 ± SE	L_3 ± SE	r^2
L. erythropterus	both	172	75.40 ± 11.67	335.21 ± 2.73	457.12 ± 10.01	0.93
	females	61	75.03 ± 17.57	346.66 ± 5.07	477.29 ± 15.64	0.95
	males	30	86.06 ± 22.0	337.47 ± 4.50	468.66 ± 18.55	0.94
L. malabaricus	both	878	78.09 ± 2.99	298.94 ± 1.72	424.92 ± 1.37	0.95
	females	159	195.37 ± 27.37	329.09 ± 4.31	423.19 ± 2.70	0.70
	males	73	100.63 ± 15.50	313.62 ± 6.91	442.67 ± 4.54	0.92
L. sebae	both	144	122.27 ± 3.22	287.28 ± 2.18	451.50 ± 3.27	0.97
	females	14	99.92 ± 17.84	277.43 ± 8.58	443.96 ± 7.54	0.99
	males	9	113.50 ± 3.98	284.83 ± 5.30	461.10 ± 4.41	0.99

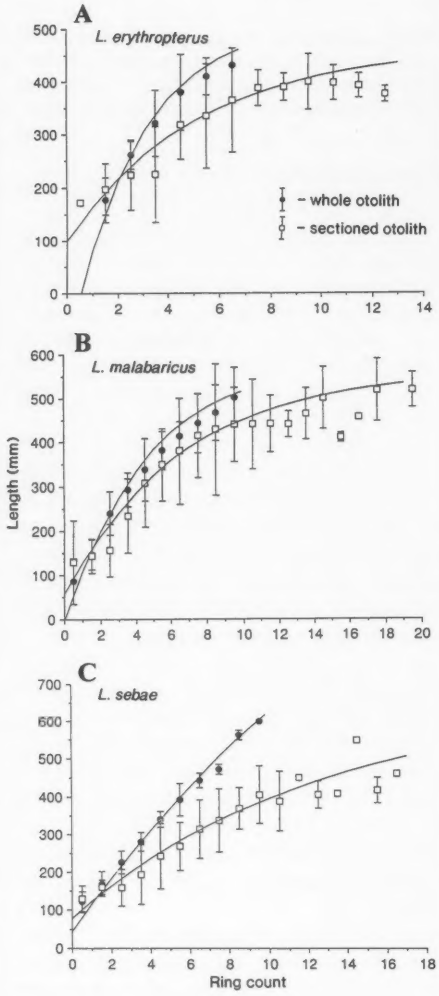

Figure 4

Plot of the mean length-at-age (±) range and the growth curves of the three species of *Lutjanus* based on ring counts in whole and sectioned otoliths.

than were other species ($P<0.05$). However, at six years of age (L_3) they were about the same size as *L. erythropterus* but were larger than *L. malabaricus* ($P<0.05$).

Relationship of ring counts in whole and sectioned otoliths with radiometric ages

There was a significant linear relationship between both whole and sectioned otolith ring counts and radiometric age (Fig. 7; $P<0.001$ in both cases). The slopes of the lines of best fit differed ($\beta=1.04 \pm 0.11$; $r^2=0.84$ for whole otolith ring counts and $\beta=1.83 \pm 0.06$; $r^2=0.87$ for sectioned otolith ring counts). Because the initial activity ratios of the *L. sebae* samples (R) were obviously greater than 0.0 in at least the whole otolith samples, these were not included in the analyses.

There was no significant difference between whole otolith ring counts and radiometric ages for all species combined ($T=53.5$; $P>0.30$, $n=15$) or for *L. malabaricus* ($T=17.5$; $P>0.15$, $n=10$). However, for all species combined we found that the sectioned ring counts were significantly greater than the radiometric age of the same fish ($T=6.5$; $P<0.001$, $n=15$). The sectioned ring counts of *L. malabaricus* were also greater than the radiometric ages ($T=2$; $P<0.005$, $n=10$).

Discussion

This is the first study to use $^{210}Pb/^{226}Ra$ activity ratios to verify the age of relatively short-lived tropical fishes. Previous studies that have used these ratios to estimate age have focussed on species that live to at least 70 years (Bennett et al., 1982; Campana et al., 1990; Fenton et al., 1991). In the Lutjanidae, natural levels of ^{226}Ra in the otoliths were high, which helped to minimize the variances in the $^{210}Pb/^{226}Ra$ activity ratio and hence the errors in the age estimates. Radiometry provided strong evidence that the rings counted in whole otoliths were the best estimate of the true age of the three lutjanids studied.

The radiometric methods we used tend to overestimate age because the assumptions concerning the otolith mass growth model and rate of incorporation of allogenic ^{210}Pb were conservative. The only conceivable mechanism that would lead to underestimation of ages radiometrically would be a significant loss of radon (^{222}Rn) from otoliths during growth (West and Gauldie, in press).

Radon is the daughter of ^{226}Ra and the only gaseous precursor of ^{210}Pb in the decay chain. Its mean lifetime is only 4.8×10^5 seconds, and its effective (physical) diffusivity in otoliths would be about 0.5×10^{-12} $m^2 \cdot s^{-1}$. Radon diffusion out of otoliths would be further retarded by adsorption to organic matter (Wong et al., 1992). Simple calculations based on the known microstructure of otoliths (Campana and Neilson, 1985) and on the existing data on radon emanation (Morawska and Phillips, 1993) show that significant loss of radon from otoliths is extremely unlikely, as previously suggested from empirical studies (Fenton and Short, 1992).

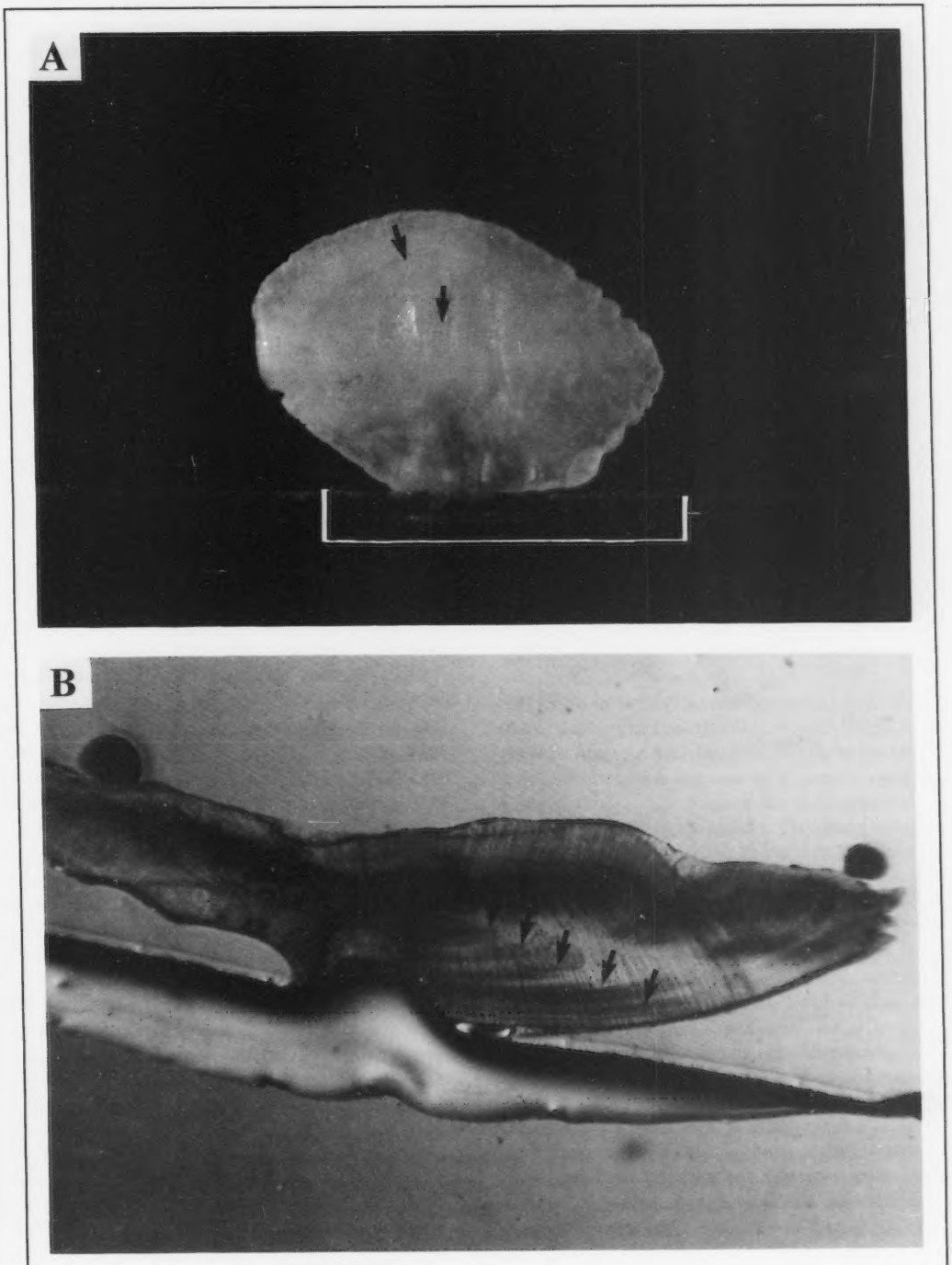

Figure 5

Photographs of an otolith of a 270-mm *L. erythropterus* showing the discrepancy between the number of rings seen by examining (**A**) the intact otolith (2 rings) and (**B**) after sectioning (5 rings). Scale=1 cm.

Why ages derived from whole and sectioned otoliths were significantly different remains unclear. The differences in ring counts increased with the size of the fish, and the slope of the regression (whole vs. sectioned ring count) was steepest for the fastest-growing species, *L. erythropterus*. The otoliths were large (up to 30 mm long, and weighing 3 g), so the daily rings during periods of reduced or variable growth of younger fish were still relatively widely spaced. Thus, what would appear as a diffuse, single hyaline zone in a whole otolith examined against reflected light may have appeared in section as a group of hyaline and opaque zones. These problems in otolith interpretation were most marked in *L. erythropterus* and led to the greatest discrepancy in ring counts.

Studies of the age and growth of *Lutjanus malabaricus* from northern Australia and the South China Sea used ring counts in vertebrae (Lai and Liu, 1974, 1979; Edwards, 1985), sectioned otoliths (Chen et al., 1984), and whole otoliths (McPherson and Squire, 1992). Their estimates were similar to the estimates we obtained from whole-otolith ageing, although *L. malabaricus* from the Great Barrier Reef appear to grow much faster and live at least one year less than those found in other areas (McPherson and Squire, 1992). However, the previous studies and our study provide different estimates of the von Bertalanffy growth parameters L_∞ and K (Table 5). These differences may have major impacts on age-structured fishery models (e.g. yield per recruit) that use these parameters to estimate optimal yield.

Lutjanus erythropterus and *L. sebae* from the Gulf of Carpentaria grew at similar rates to those reported from other parts of northern Australia (Ju et al., 1988; McPherson and Squire, 1992) and elsewhere within their range (Druzhinin and Filatova 1980; Yeh et al., 1986; McPherson and Squire, 1992). However, the growth of *L. sebae* in the Gulf of Carpentaria did not decline as they approached the maximum age observed. This may have been caused by an error in the ring count in otoliths of older fish or because the older age classes were not caught in the trawls. The maximum size of L. sebae in Australian waters has been reported to be between 1.0 and 1.4 m (Allen,

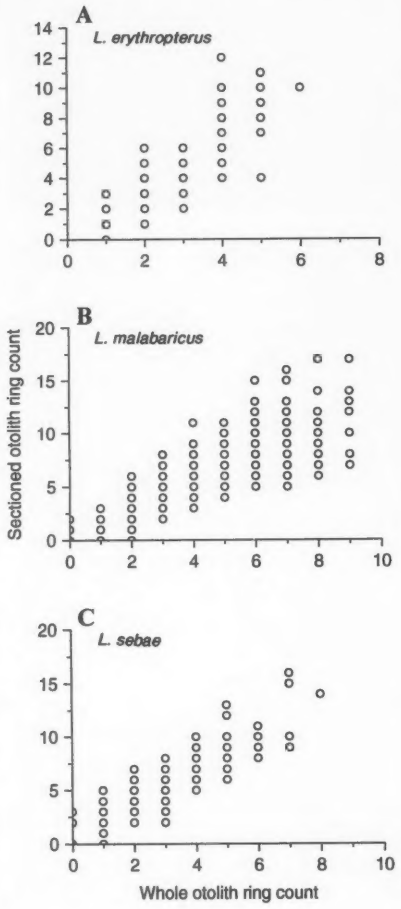

Figure 6

Plot of the relationship between whole and sectioned otolith ring counts of the three species of *Lutjanus*.

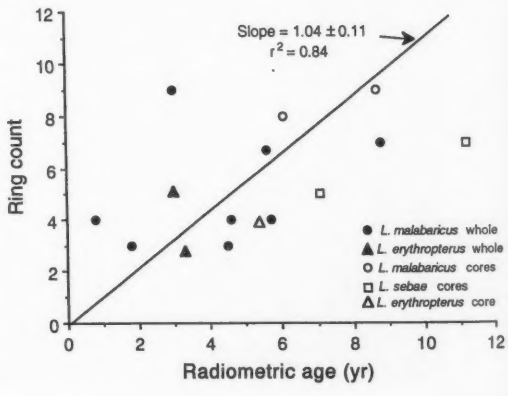

Figure 7

The relationship between radiometric age (yr) and whole otolith ring counts of all samples (except *L. sebae* whole otolith samples).

Table 5

Von Bertalanffy growth parameters of tropical *Lutjanus* from northern Australia and elsewhere within their range (W=whole otoliths; S=sectioned otoliths; V=vertebrae; U=urohyal; Sc=scales).

Species	Locality	Sex	Method	K	L_∞	Maximum age	Reference
L. erythropterus	Gulf of Carpentaria	Both	W	0.30	565	6	Present study
	Great Barrier Reef	F	W	0.44	500	7	McPherson and Squire (1992)
		M	W	0.41	500	7	McPherson and Squire (1992)
	Northwest Shelf	Both	V	0.21	603	7	Ju et al. (1988)
L. malabaricus	Arafura Sea	Both	V	0.17	707	10	Edwards (1985)
		Both	V	0.12	790	8	Lai and Lui (1979)
	Gulf of Carpentaria	Both	W	0.22	592	9	Present study
	N.W. Australia	Both	V	0.13	768	8	Lai and Lui (1979)
		Both	V	0.25	715	10	Chen et al. (1984)
	S. China Sea	Both	V	0.14	790	11	Lai and Lui (1974)
	Great Barrier Reef	F	W	0.23	696	7	McPherson and Squire (1992)
		M	W	0.18	820	7	McPherson and Squire (1992)
	Vanuatu	Both	S	0.31	600	—	Brouard and Grandperrin (1984)
L. sebae	Gulf of Aden	Both	Sc	0.16	660	11	Druzhinin and Filatova (1980)
	Gulf of Carpentaria	Both	W	0.06	1483	9	Present study
	N.W. Australia	Both	V	0.13	678	10	Yeh et al. (1986)
	Great Barrier Reef	F	W	0.18	851	8	McPherson and Squire (1992)
		M	W	0.15	736	8	McPherson and Squire (1992)
L. vittus	N.W. Australia	F	U	0.37	267	7	Davis and West (1992)
		M	U	0.22	346	8	Davis and West (1992)

1985; Grant, 1985) or 16 to 22 kg (Grant, 1985; Allen and Swainston, 1988), which is much greater than we recorded (5 kg). This species may, therefore, live more than 10 years. Indeed, large *L. sebae* (over 800 mm) from the Great Barrier Reef are known to live on deep coral reefs at depths greater than 60 m[1]; the deepest part of the Gulf of Carpentaria is only 55 m. This suggests that fish may move from this region as they grow.

Our radiometric ageing results have several important implications beyond the verification of the age structure of each species. First, they demonstrated that for species that have a high otolith ^{226}Ra specific activity, $^{210}Pb/^{226}Ra$ activity ratios can be used to age fish as young as 3 years with accuracy. Previously these radioisotopes have only been used to age long-lived species (>10 yr; Bennett et al., 1982; Campana et al., 1990; Fenton et al., 1991). Other radioisotope pairs ($^{228}Th:^{228}Ra$) have been used to age short-lived tropical species (Campana et al., 1993), but these are only useful for fish up to 5 years old because of the short half-life of Th–228.

Second, for relatively short-lived species, radiometric ageing of whole otoliths and cores using a single-phase linear model of otolith mass growth rate gave similar results. Campana et al. (1990) and Smith et al. (1991) argued that new material accreting to the outer surface of the otolith may not accrete ^{226}Ra in similar specific activities to the juvenile (t=0). This would invalidate the use of a simple otolith mass growth model to interpret the radiometric data for otoliths of postjuvenile fish. However, even with a single-phase linear mass growth model (a two-phase model would have reduced the age estimates), we were able to verify that the ring counts in whole otoliths were a more accurate measure of the true age than counts from sectioned otoliths (in accord with core radiometric ages). However, we agree with Smith et al. (1991) that otoliths should be cored for radiometric ageing, if possible, which would avoid the use of an otolith mass growth model.

The third point that arises from our analyses relates to the ratio of allogenic to radiogenic lead in *Lutjanus* otoliths. We set the uptake activity ratio value at zero (R=0.0) because higher values would have lowered the age estimates (e.g. Smith et al., 1991). However, from the stable lead/barium ratios and the high age estimates of two of the *L. sebae* samples (2068 and 2069) it appears that, at least for this species, the juveniles may be taking up more allogenic ^{210}Pb than the adults (Fenton and Short, 1992). There was no systematic increase in the Pb/Ba mass ratios of *L. malabaricus* and there is insufficient data for *L. erythropterus* to be conclusive (Fig.

[1] Williams, D. Australian Institute of Marine Science, PMB No. 3, Townsville 4810, Queensland, Australia. Personal commun., 1993.

3). However, for lutjanids it appears that a Pb/Ba mass ratio <0.2 probably indicates that the assumption of a low initial activity ratio (R) is valid, whereas the three samples where the Pb/Ba mass ratio is 0.3–0.6 indicate that the assumption of low R may be invalid. The Pb/Ba ratios are, therefore, a useful test of the validity of the low R assumption.

Finally, this appears to be the first instance where radiometric methods are more consistent with whole-otolith ages rather than sectioned-otolith ages. All previous radiometric studies of fish from temperate and subtemperate waters have verified section counts (Bennett et al., 1982; Campana et al., 1990; Fenton et al., 1990, 1991; Smith et al., 1991). The metabolic effects of the annual cycle of inorganic and organic deposition in otoliths may be more pronounced in these environments resulting in clear annuli in otoliths of fish from more temperate regions.

Conclusions

This study has shown that radiometry using $^{210}Pb/^{226}Ra$ activity ratios in both whole and cored otoliths can accurately estimate the ages of fish as young as 3 years. Stable lead:barium mass ratios were used to identify samples that may invalidate the assumption of constant uptake of allogenic lead (R=0). For the lutjanids examined, ring counts in sectioned otoliths were shown to overestimate fish ages. Methods such as marginal increment analysis do not verify that the ageing method used is accurate unless the pattern is demonstrated to be consistent for all age classes. This indicates that tropical fish should be aged by two independent methods where possible to help minimize possible ageing errors.

Acknowledgments

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Age and growth estimates for the dusky shark, *Carcharhinus obscurus*, in the western North Atlantic Ocean

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Abstract.—Age and growth of the dusky shark, *Carcharhinus obscurus*, was estimated from bands in the vertebral centra of 122 individuals and from length-frequency data from 341 individuals. The von Bertalanffy growth function parameters from the vertebral analysis were considered more robust (L_∞=373, K=0.038, t_0=–6.28, male; L_∞=349, K=0.039, t_0=–7.04, female). Comparison of male and female growth curves generated from vertebral data indicate a statistically significant difference; however, these differences are due primarily to larger sizes attained by adult females. Estimates of age at maturity indicate that dusky sharks follow the typical carcharhinid pattern of slow growth and late age at maturity. The size at maturity is reported at 231 cm FL and 235 cm FL for males and females, respectively. These lengths correspond to approximately 19 years for males and 21 years for females. The oldest fish aged from vertebrae was a 33+ year-old female.

Sharks have become increasingly important in U.S. commercial fisheries in the western North Atlantic Ocean in recent years. U.S. landings of large coastal sharks, represented primarily by several species in the family Carcharhinidae, increased from 135 to 7,122 metric tons (t) from 1979 to 1989 (Anon., 1993). Musick et al. (1993) reported that annual recreational catches are estimated to be 35,000 U.S. tons and related annual mortality is over 10,000 U.S. tons (9,074 t). As a group, sharks tend to exhibit slow growth, late age at maturity, and low fecundity (Holden, 1973). As a consequence of these life history characteristics, recruitment in sharks is directly dependent on stock size (Holden, 1973). This direct relationship means that elasmobranchs may not be able to recover readily from overexploitation (Holden, 1973).

The dusky shark, *Carcharhinus obscurus*, is part of the species complex presently managed under the Secretarial Shark Fisheries Management Plan (FMP) for the Atlantic Ocean (Anon. 1993). Currently, dusky sharks are harvested in commercial fisheries off the southeastern United States and in the Gulf of Mexico. Recreational fishermen off the northeastern United States also catch dusky sharks (Casey and Hoey, 1985; Musick et al., 1993). The shark FMP (Anon. 1993) details the need for accurate life history information on individual species taken in the shark fishery. Proper management at the species level requires specific information on age and growth.

The dusky shark is a common coastal pelagic species with a worldwide distribution in temperate and tropical waters (Compagno, 1984). In the western North Atlantic, it ranges from as far as Banquereau Bank off Nova Scotia, Canada, to southern Brazil, including the Gulf of Mexico and Caribbean Sea (Hoey, 1983; Compagno, 1984). Tagging studies show dusky shark movements from southern New England to Yucatan, Mexico (Casey et al.[1]; Hoey, 1983).

Age and growth studies of large sharks are difficult because many species are highly migratory, making them available for only short seasonal periods, and different elements of the population segregate spatially by size and sex (Hoenig and Gruber, 1990). In addition, the large size attained by adults makes them difficult to sample. Recent literature has discussed the benefits of growth and longevity estimates attained from tag and recapture

[1] Casey, J. G., H. L. Pratt Jr., and C. E. Stillwell. 1980. The shark tagger summary. Newsletter of the Coop. Shark Tagging Program. U.S. Dep. Commer., Northeast Fish. Sci. Cent., Natl. Mar. Fish. Serv., 28 Tarzwell Rd., Narragansett, RI, 02882-1199.

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Fishery Bulletin 93:116–126 (1995).

studies (Casey and Natanson, 1992). These data are not available for the dusky shark nor is validation of vertebral band periodicity. Previous attempts to age the dusky shark were based on limited data and were inconclusive (Lawler, 1976; Hoenig, 1979; Schwartz, 1983). We have attempted to strengthen age estimates of *C. obscurus* by using vertebral band counts together with marginal increment analysis and by using comparisons with length-frequency data. With the von Bertalanffy growth function thus derived, we estimate age at maturity and longevity for this species.

Materials and methods

Data and vertebral samples from dusky sharks were obtained between 1963 and 1993 from research cruises, sport fishing tournaments, and commercial shark fishermen from Cape Cod, Massachusetts, to off the east coast of Florida. Vertebral samples were taken in all months except January, March, and November.

Length measurements

Total and fork lengths were measured to the nearest centimeter (cm) for each specimen. Fork length (FL) was measured from the tip of the snout to the fork of the tail. Total length (TL) is defined as the distance from the snout to a point on the horizontal axis intersecting a perpendicular line extending downward from the tip of the upper caudal lobe to form a right angle (Kohler et al.[2]). All lengths used are fork lengths unless otherwise noted. FL can be converted to TL by using the regression equation:

$$FL = 0.8352\,(TL) - 2.2973.\ [r^2 = 0.99,\ n = 167]$$

Vertebral samples

Vertebral samples were taken from above the branchial chamber. Sections of vertebral columns were trimmed of excess tissue and then frozen or preserved in 70% ethanol (Casey et al., 1985).

Two vertebrae from each specimen were processed histologically following Casey et al. (1985), with the exception of the use of RDO (DuPage Kinetics) for decalcification. All vertebral sections were cut sagittally through the focus to a thickness of 80–100 microns, stained with Harris hematoxylin, and mounted in glycerin jelly (Humason, 1972).

Bands in the vertebra were counted from an image projected on a Summagraphics MM-1812 digitizing tablet (Skomal, 1990). Measurements from the focus to growth bands at points along the internal corpus calcareum were digitized directly into an IBM PC-XT computer. The radius of each centrum was measured from the focus to the distal margin of the intermedialia along the same diagonal as the band measurements. Annual growth marks were defined following Casey et al. (1985) for the sandbar shark, *Carcharhinus plumbeus*, where the annual mark is defined by a wide translucent zone that traverses the intermedialia and continues into the corpus calcareum as an opaque band.

Vertebral sections from 171 dusky sharks were prepared. Bands in the same centrum section were counted at least once by each of four investigators to verify that the band counts were repeatable. Sections were considered unreadable if bands could not be discerned in accordance with the above definition. If two readers considered the section unreadable, the sample was eliminated from the final analysis.

Counts were accepted if two or more readers agreed. The individual ring measurements for all readers in agreement were then averaged. If two readers agreed on one count and two on another for the same specimen, the higher count was accepted. Specimens where there was no initial agreement were recounted until two of the investigators reached a consensus or the sections were discarded.

The relationship between vertebral radius (VR) and FL was calculated to determine the most appropriate method for back calculation of the size-at-age data (Ricker, 1969). The FL to VR relationship was linear but did not pass through the origin. Therefore, the Lee method was considered more appropriate (Ricker, 1969):

$$l = a + (b \times s),$$

where l = the length of fish when the vertebra was obtained;
a = the intercept on the length axis;
b = the slope of the line; and
s = the total vertebral radius.

A von Bertalanffy growth function (VBGF) was fitted to the data by using the following equation (von Bertalanffy, 1938):

$$L_t = L_\infty\left(1 - e^{-k(t-t_0)}\right),$$

where L_t = predicted length at time t;
L_∞ = mean asymptotic fork length (of the fish);
K = a growth rate constant (yr^{-1}); and
t_0 = the theoretical age at which the fish would have been zero length.

[2] Kohler, N. E., J. G. Casey, and P. A. Turner. Length-weight relationships for 13 Atlantic sharks. Unpubl. manuscr.

Growth in length data were analyzed by using FISHPARM, an IBM PC compatible program (Prager et al., 1987), which implements Marquardt's algorithm for nonlinear least squares parameter estimation (Marquardt, 1963).

Bernard's (1981) multivariate analysis for comparing growth curves was employed to test the hypothesis that male and female vertebral growth curves were the same. This method also determines which of the von Bertalanffy parameters are the most statistically significant cause of any differences in growth.

Marginal increment analysis

Validation, the confirmation of the temporal meaning of the growth increment (Brothers, 1983), is difficult to attain for large pelagic species and was attempted by using marginal increment analysis. The marginal increment ratio (MIR) (Skomal, 1990) was calculated by using the following equation:

$$MIR = (VR - R_n)/(R_n - R_{n-1}),$$

where VR = the vertebral radius;
R_n = the last complete band; and
R_{n-1} = the next to last complete band.

Mean MIR was plotted against month to locate periodic trends in band formation. The MIR relates the edge formation to the width of the previous completed band, which corrects for differences in band width between small and large fish.

Length frequency

Length-frequency distributions were analyzed by using Shepherd's (1987) model. The sample was separated by sex and calculations were made at 3-cm intervals. Initial values of L_∞ and K, based on biological parameters obtained from the literature (Springer, 1960; Compagno, 1984) were entered into the program which was then rerun until the highest score function was attained. The L_∞ and K associated with this score function were used to calculate t_0 by using the following equation:

$$t_0 = t + (1/K)\,(\ln[L_\infty - L_t]/L_\infty),$$

where t_0 = 0 (birth);
L_t = mean size at birth;
K = the von Bertalanffy growth constant; and
L_∞ = the mean asymptotic fork length.

Longevity

Estimates of longevity were obtained by using tag and recapture data. Data on eight recaptured dusky sharks at liberty for greater than 10 years were examined. Age at tagging was assigned from the size estimate provided at the time of release. This estimated age was based on growth curves derived from vertebrae. The number of years at liberty were then added to estimate age at recapture.

Results

Vertebral samples

Of the 171 processed vertebra, 36 (21.0%) were considered unreadable. Initial agreement by two or more readers was reached on 89 specimens. The remaining 50 sections were recounted by two of the investigators. A consensus was reached on 37 of those recounted and the rest were discarded as unreadable. Six were then eliminated for having no information on sex. The remaining 120 (70.2%) consisted of 53 male and 67 female specimens ranging in size from a 73 cm FL neonate to a 296 cm FL adult female. The FL-VR regression showed a linear relationship:

$$FL = 12.82(VR) + 24.99\ [n = 114;\ r^2 = 0.99]\,.$$

The FL to VR relationship was significantly different between the sexes for all fish combined (ANCOVA, $P<0.05$). However, this was due to three large females whose removal from the analysis altered the curves and showed the males and females to be statistically indistinguishable ($P<0.05$) (Fig. 1). We chose to use the combined relationship without those three samples.

Back-calculated as compared with empirical length-at-age data show a smaller estimated size for fish of younger ages, when calculated from the vertebrae of the older fish, indicating the presence of a slight Lee's phenomenon for both sexes (Table 1). Lee's phenomenon was more pronounced in females and increased with age.

The MIR data showed a distinct, periodic trend of increasing increment growth from April through June (female) or July (male); after this peak there was a slight decrease and apparent leveling (Fig. 2). The decrease in incremental growth is not large enough to indicate a double band formation. The graph suggests that an annual winter band is formed between September and April. This band can be visible by February in males; no data were available for females. The time of annulus formation cannot

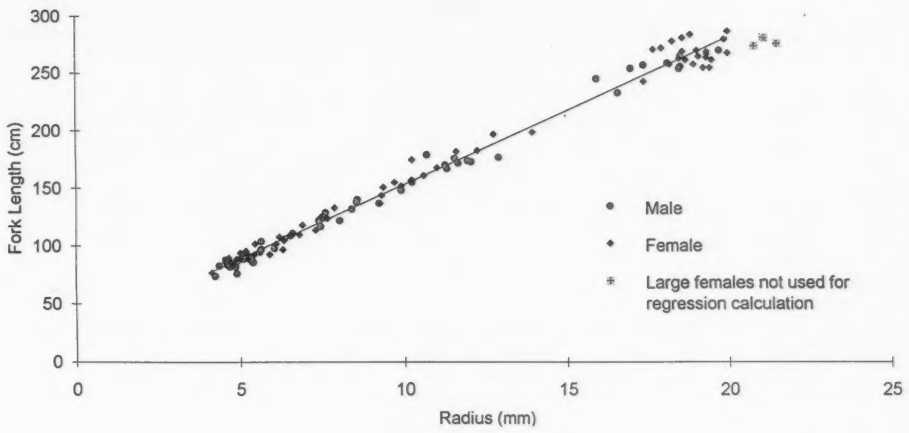

Figure 1

Relationship between vertebral radius (cm) and fork length (cm) for male and female dusky sharks, *Carcharhinus obscurus*.

be further established owing to a lack of winter samples. January was used as the month of band formation for the assignment of age classes (Casey et al., 1985).

Back-calculated length at first band (80.2 cm FL male; 85.8 cm FL female) corresponded closely to the known size at birth of 85–100 cm TL (Castro, 1983; Compagno, 1984). The first winter band would have formed after approximately six months growth (assuming January deposition and spring parturition), and the following bands represented annual growth (Branstetter, 1987). The oldest female in the sample was 33+ years and the oldest male, 25+ years.

The parameters of the VBGF determined from the back-calculated data were similar to known life history characteristics except that the predicted L_∞ for males was higher than that for females (Table 2). Those samples that were neonates with no visible birthmark were excluded from the VBGF analysis. Therefore, only 114 samples (47 male and 67 female) were included in the final calculations. The t_0 and K values appear similar between the sexes (Table 2). However, the male and female growth curves are significantly different ($P<0.05$) based on Bernard's (1981) multivariate analysis (Table 3). The results indicated that the differences were caused by the t_0 and L_∞ values (in order of significance).

The reported size at maturity for the dusky shark is 231 cm FL and 235 cm FL for males and females, respectively. These lengths correspond to 19 years for males and 21 years for females based on the vertebral growth curves (Table 4).

Length frequency

Length observations from a total of 208 female and 133 male dusky sharks were used to calculate von Bertalanffy parameters by using length-frequency analysis. Samples were obtained from 1961 to 1987 for the months May through November. Because of small yearly sample sizes, data for all years were combined by month.

A comparison of the VBGF parameters from the length-frequency analysis [LF] with those derived from vertebral analysis (Table 2) shows that the L_∞ and t_0 values from the vertebral analysis for females were lower than those derived from the length-frequency analysis, and that the K value for females was basically the same for both data sets. The length-frequency analysis for males results in a lower L_∞ than that from the vertebral analysis and in higher t_0 and K values. The VBGF differences in both sexes are not large and both curves indicate late age at maturity (males: 25 yr [LF], 19 yr [vertebral]; females: 16 yr [LF], 21 yr [vertebral]) and slow growth (males: K=0.049 [LF], 0.038 [vertebral]; females: K=0.040 [LF], 0.039 [vertebral]) (Fig. 3). The von Bertalanffy parameters for the sexes combined are shown for comparison (Table 2).

Longevity

Tagging records from NMFS Cooperative Shark Tagging Program show that 6,067 dusky sharks were tagged and 131 recaptured between 1962 and 1992.

Table 1

Back-calculated and observed size-at-age data for male and female dusky shark, *Carcharhinus obscurus*.

	Male Ring (age in years)										
	Birth	6 months	0	1	2	3	4	5	6	7	8
Back-calculated											
$\bar{X}$	80.2	87.3	94.1	103	113.9	124.4	132	140.5	149.7	157.7	163.8
SD	5.6	5.5	6.2	7	9.2	10.6	10.7	11.7	12.7	12.6	10.5
n	47	34	30	28	25	21	20	20	19	17	
Observed											
$\bar{X}$	87.7	100.5	123.5	116.7	124.5	138		148	173.5	164	
SD	5.1	53.		2.1	5.5	4.9	0		0	0.7	7.9
n	13	4		2	3	4	1		1	2	3

	Male Ring (age in years)										
	9	10	11	12	13	14	15	16	17	18	19
Back-calculated											
$\bar{X}$	161.7	179.2	188.6	196.1	202.4	209.8	216.1	220.5	226.5	232.3	237.9
SD	11.6	12.3	10.4	11.8	11.7	13.5	13	11.4	11.7	12.4	12.5
n	14	13	11	10	10	10	10	9	9	8	8
Observed											
$\bar{X}$	172	177.5	177				233		245		260.5
SD	0	2.1	0				0		0		9.2
n	1	2	1				1		1		2

	Male Ring (age in years)										
	20	21	22	23	24	25	26	27	28	29	30
Back-calculated											
$\bar{X}$	246.4	252.6	254.9	256.6	252.6	255.2					
SD	14.5	13.2	15.1	16.1	0	0					
n	6	6	4	3	1	1					
Observed											
$\bar{X}$		256.5	256	263.5		265					
SD		3.5	0	9.2		0					
n		2	1	2	1						

	Female Ring (age in years)										
	Birth	6 months	0	1	2	3	4	5	6	7	8
Back-calculated											
$\bar{X}$	85.8	92.3	99.3	107.4	118.8	128.6	136.2	143.7	150.5	157.8	164.8
SD	5.1	4.5	5	6.3	8.4	9.4	9.5	8.5	8.8	9.4	9.9
n	67	55	52	50	48	45	41	38	35	33	33
Observed											
$\bar{X}$	91.8	104.3	106.5	107	122.3	134.8	138.3	161	154.5		
SD	4.5	7.2	6.4	2.8	3.8	16.8	26	12.5	3.5		
n	12	3	2	2	3	4	3	3	2		

Table 1 (continued)

	Female Ring (age in years)										
	9	10	11	12	13	14	15	16	17	18	19
Back-calculated											
$\bar{X}$	171.5	178.8	186.3	192.9	199.8	206.2	212.4	216.7	222.6	228.4	233.4
SD	10.2	9.6	10.8	10.9	12.4	12.9	13.3	10.5	11.6	12.3	12.6
n	33	30	28	28	28	28	28	27	27	27	27
Observed											
$\bar{X}$	183	190					262				281
SD	15.5	9.9					0				0
n	3	2					1				1

	Female Ring (age in years)										
	20	21	22	23	24	25	26	27	28	29	30
Back-calculated											
$\bar{X}$	237.9	242.1	246	149.8	252.9	256.4	258.6	262.9	265.4	266.2	265.1
SD	12.3	12.1	12.5	12.2	12.6	13.5	11.9	13.3	11.8	15.2	12.7
n	26	24	23	22	20	16	15	13	9	5	4
Observed											
$\bar{X}$	253.4	263	284	156.5	161.5	274	266.5	272	270.8	281	262
SD	14.8	0	0	2.1	9	0	16.3	10.5	6.5	0	0
n	2	1	1	2	4	1	2	4	4	1	1

	Female Ring (age in years)										
	31	32	33	34	35	36	37	38	39	40	41
Back-calculated											
$\bar{X}$	267.9	277.1	291.4								
SD	15.2	16.6	0								
n	3	2	1								
Observed											
$\bar{X}$	269	269	276								
SD	0	0	0								
n	1	1	1								

Eight of these fish were at liberty from 10.1 to 15.8 years. Estimated ages at tagging were based on the vertebral growth curve and ranged from birth to 27 years. The best example of longevity came from a dusky shark that was tagged at an estimated 27 years (260 cm FL) and was recaptured 12 years later at an estimated age of 39 years (Table 5).

Discussion

In the present study, vertebral data and length-frequency data were independently analyzed to derive estimates of von Bertalanffy growth parameters for the dusky shark. Because of the differences between the methods and their sensitivity to the data used to calculate the VBGF parameters, each method produced slightly different growth curves and, therefore, different estimates of age at maturity and longevity (calculated based on maximum reported size). The length-frequency estimates obtained from the dusky shark data are probably somewhat biased owing to limitations of the data and properties of the length-frequency model (Majkowski et al., 1987; Shepherd

et al., 1987; Natanson, 1990). As a slow-growing, long-lived species, the dusky shark may have overlapping lengths at age which may obscure length modes and bias the estimates of model parameters (Rosenberg and Beddington, 1987; Shepherd et al., 1987). The vertebral method is therefore considered the more robust method and the length-frequency parameters are used for comparison only.

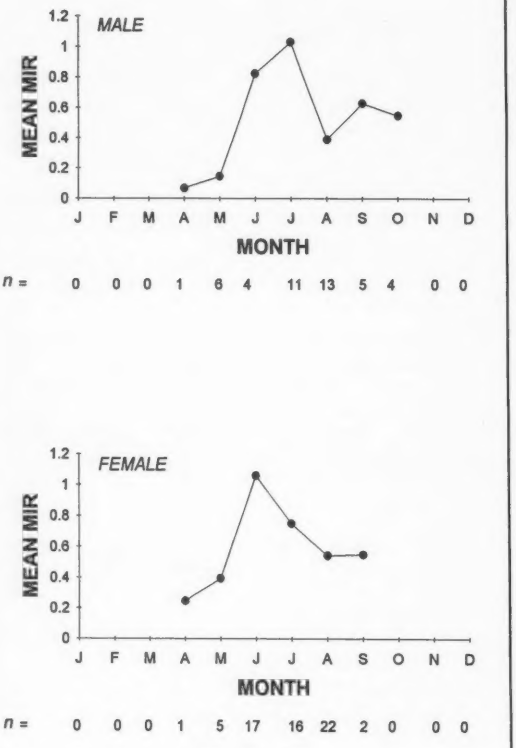

Figure 2

Mean vertebral marginal increment ratios (MIR) by month for each of four readers for the dusky shark, *Carcharhinus obscurus*. Number of samples used to calculate the means for each month are located below the figure.

Yoccoz (1991) has brought up questions as to the validity of judging biological significance based on statistical tests. He suggests that statistical significance is not necessarily indicative of biological significance; this appears to be the case with the dusky shark. The statistically significant differences shown between male and female dusky shark vertebral growth curves may not reflect biological differences. Examination of the length-at-age data suggests that biologically the differences between male and female vertebral curves are small. The age and size at maturity differ by only two years and five centimeters for males and females (Table 4). Females are presumed to grow ultimately to a larger size than males. This means that either growth slows in males after maturity or that males do not live as long as females.

The vertebral VBGF derived in this study is very similar to the curve attained by Hoenig (1979) for combined sexes for the ages under consideration (birth to 33 years) but is different from data presented by Lawler (1976) and Schwartz (1983). Hoenig's (1979) parameter values for the VBGF have a slightly higher L_∞ and t_0 and lower K than parameters derived from vertebral analysis in the present study (Table 2). Lawler (1976), using vertebral analysis to determine the age of female dusky sharks, obtained VBGF-parameter values markedly different from the present study (Table 2). The L_∞ in his study is more than twice as large as the L_∞ reported here and his K value suggests a much slower growth rate. These two factors combine to make Lawler's (1976) curve appear as a straight line from birth to 34 years.

Table 2

The von Bertalanffy parameters derived in this study compared to those derived in Hoenig's (1979) study of male and female dusky sharks and Lawler's (1976) study of female dusky sharks, *Carcharhinus obscurus*.

	Male				Female				Combined			
Parameters	L_∞	K	t_0	n	L_∞	K	t_0	n	L_∞	K	t_0	n
This study:												
Vertebrae	373	0.038	–6.28	47	349	0.039	–7.04	67	352	0.040	–6.43	120
Length-frequency	293	0.049	–5.99	133	392	0.040	–5.34	208	296	0.062	–4.68	350
Hoenig, 1979									385	0.034	–5.99	22
Lawler, 1976					732	0.014	–6.7	13				

Lawler's (1976) L_∞ is unrealistically high when compared with the maximum reported size from the literature (308 cm FL [Springer, 1960]) and with those derived in both Hoenig's (1979) study and the present one. Schwartz (1983) also used vertebral bands in an attempt to age the dusky shark. In general, his back-calculated size-at-age data (Schwartz, 1983; Table 4) are lower than those in the present study. His estimates of size at birth are much lower than reported sizes at birth, suggesting that prebirth bands may have been counted (Casey et al., 1985). Marginal increment data from the present study do not support Schwartz's (1983) hypothesis of a much faster growth rate with two bands per year based on his marginal increment data.

Tagging data combined with vertebral estimates indicate that the dusky shark can live for at least 40 years. The largest reported dusky shark in the literature was a 308 cm FL female (Springer, 1960) which corresponds to 51 years based on our vertebral VBGF. Tag and recapture data on the large dusky shark at liberty for 12 years add credence to the vertebral longevity estimate by verifying that these fish do live up to 40 years. Thus, the dusky shark appears to be a long-lived, late-maturing species, exhibiting a pattern typical of carcharhinids. The female bull shark, *C. leucas*, can live over 24 years and does not reach maturity until at least 18 years of age (Branstetter and Stiles, 1987). Tagging evidence has shown that the sandbar shark, *C. plumbeus*, can reach ages of at least 40 years and may not mature until 29 years of age (Casey and Natanson, 1992). The lemon shark, *Negaprion brevirostris*, which reaches at least 20 years of age and does not reproduce until 11 to 13 years, also follows this pattern (Brown and Gruber, 1988). Preliminary estimates on the age of the bronze whaler, *C. brachyurus*, indicate that males do not mature for 13 to 19 years and females for 19 to 20 years. Both may attain ages over 30 years (Walter and Ebert, 1991). The blue shark, *Prionace glauca*, appears to be atypical of most carcharhinids in having a relatively fast growth rate and a short life-span (13–16 years) (Skomal, 1990). A recent recapture from an Australian school shark, *Galeorhinus australis*, after 42 years at liberty indicates that the galeorhinids also follow this pattern.[3]

Casey and Natanson's (1992) study on the age of the sandbar shark (*C. plumbeus*) showed that in a

Table 3

Results of comparison of the vertebral von Bertalanffy growth equations for male and female dusky sharks, *Carcharhinus obscurus*, using Bernard's (1981) multivariate analysis. Shown is the estimated variance-covariance matrix (S).

	L_∞	K	t_0	
L_∞	131.9769	–0.02831	–3.16979	
K		0.0000065	0.00077	$= S$
t_0			0.115042	

[3] Olsen, A. H. Newton 5074, South Australia. Personal commun., Jan. 1994.

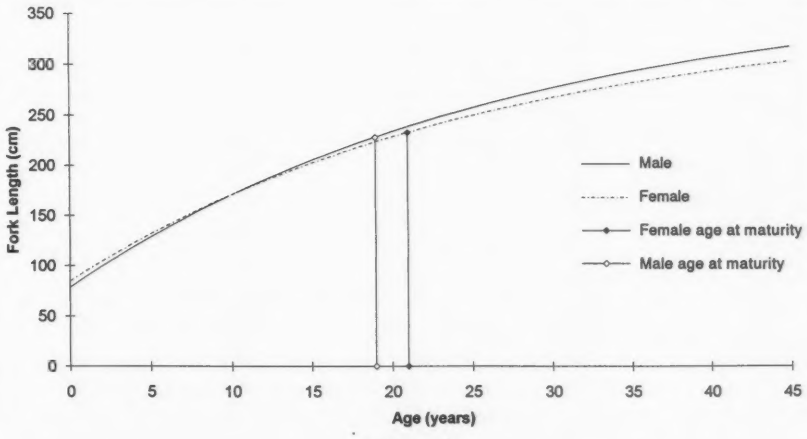

Figure 3

Von Bertalanffy growth curves generated from vertebral data for male and female dusky sharks, *Carcharhinus obscurus*.

species where good tag and recapture data are available (i.e. where specimens are measured at both tagging and recapture, properly identified, and there is sufficient sample size), a better estimate of longevity is produced than from hardpart analysis. They concluded that the initial growth curve based on vertebrae for the sandbar shark had severely underestimated the age at maturity and maximum age and had overestimated the growth rate (Casey et al., 1985). At the present time, the vertebral growth curve

Table 4

Size at age of the dusky shark, *Carcharhinus obscurus*, calculated from the von Bertalanffy growth equations. Size at age closest to maturity is in bold type.

Years	Length-frequency male	Length-frequency female	Length-frequency combined	Vertebral male	Vertebral female	Vertebral combined
Birth	75	75	75	78	84	81
1	95	100	100	89	95	92
2	104	111	112	100	105	102
3	113	122	123	110	114	112
4	122	133	134	119	123	121
5	130	143	143	129	132	131
6	138	153	153	138	140	139
7	145	162	161	146	148	148
8	152	171	169	155	156	156
9	159	180	177	163	163	164
10	166	188	184	170	171	171
11	172	196	191	178	177	178
12	177	204	197	185	184	185
13	183	211	203	192	190	192
14	188	218	209	199	197	198
15	193	225	214	205	202	204
16	198	**232**	219	211	208	210
17	203	238	223	217	214	216
18	207	244	228	223	219	221
19	211	250	**232**	**228**	224	226
20	215	255	**236**	234	229	**231**
21	219	261	239	239	**233**	236
22	222	266	243	244	238	241
23	226	271	246	249	242	245
24	229	276	249	253	246	250
25	**232**	280	252	258	250	254
26	235	294	254	262	254	257
27	238	289	257	266	258	261
28	240	293	259	270	261	265
29	243	297	262	274	264	268
30	245	300	264	277	268	272
31	247	304	266	281	271	275
32	250	307	267	284	274	278
33	252	311	269	287	277	281
34	254	314	271	291	280	284
35	256	317	272	294	282	286
36	257	320	274	297	285	289
37	259	323	275	299	287	292
38	261	325	276	302	290	294
39	262	328	277	305	292	296
40	264	331	279	307	294	298
41	265	333	280	310	296	301
42	266	335	281	312	298	303
43	268	338	282	314	300	305
44	269	340	282	316	302	307
45	270	342	283	318	304	308

Table 5

Tag and recapture data used to obtain longevity estimates for the dusky shark, *Carcharhinus obscurus*. N/A = not available.

		Tagging			
Tag number	Sex	Fork length (cm) (estimated)	Age (estimated)	Time at liberty (years)	Total age (years)
63647	N/A	260	27 yr	12	39
YR200X	N/A	84	6 months	15.8	16.3
42204	F	130	4+ yr	11.9	16+
21367	F	73	Birth	11.6	11.6
48478	N/A	116	3+ yr	10.9	13.9+
39360	F	111	2+ yr	10.1	12.1+
F66 8802	M	73	Birth	11.8	11.8
37913	F	122	4 yr	14.5	18.5

is the best estimate available for the dusky shark. However, it should be kept in mind that the vertebral growth curve generated for the dusky shark may also underestimate age at maturity and maximum age.

Acknowledgments

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Maturity, spawning, and seasonal movement of arrowtooth flounder, *Atheresthes stomias*, off Washington

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Abstract.—Arrowtooth flounder, *Atheresthes stomias*, were sampled for macroscopic maturity stage, and females were subsampled for gonosomatic index (GSI) from commercial trawl landings from July 1991 to July 1992 and during the NMFS triennnial bottom trawl survey from September to November 1992. Arrowtooth flounder are batch spawners and spawn from fall to winter off Washington at depths of at least 366 m (200 fm). Hydrated oocytes were first seen in September 1991 when mean GSI for yolked oocyte-bearing females approximately doubled. Postovulatory follicles were present in all macroscopic stages of mature ovaries sampled for histology in late December 1991. By March 1992 all mature females were spent; females with hydrated oocytes were again seen in November 1992. Arrowtooth flounder show a group-synchronous pattern of oocyte development. Catch rates from trawl logbook data suggest that the mature population migrates seasonally, from depths of about 183 m (100 fm) in summer to depths exceeding 475 m (260 fm) in winter. Fork length at which 50% of fish were sexually mature ($L_{50\%}$) calculated from survey data was 28.0 cm for males and 36.8 cm for females, similar to estimates from Washington commercial trawl data but less than estimates from Oregon in the 1970's. Female $L_{50\%}$ varied seasonally. Problems of bias associated with sampling commercial trawl landings for size at maturity are discussed.

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Arrowtooth flounder, *Atheresthes stomias*, is a large, predatory flatfish ranging from the Bering Sea to central California (Hart, 1973). It is estimated to be the single most abundant species in the Gulf of Alaska and has shown significant increases there in both population numbers and biomass.[1] Arrowtooth flounder is typically not considered a high-value species in the Washington trawl fishery, but recent landings have been substantial. In 1990–92 more arrowtooth flounder were landed from areas off Washington than any other groundfish species except Pacific whiting,[2] and management interest in arrowtooth flounder has intensified. Little detailed information is available on arrowtooth flounder in the eastern Pacific Ocean. Kabata and Forrester (1974) studied parasitic infection of arrowtooth flounder off British Columbia and noted the population length and age structure as well as diet, but did not examine maturity. Pertseva-Ostroumova (1960) examined *Atheresthes* reproduction and larval development in the Bering Sea, but spawning data on *A. stomias* was limited and conclusions regarding time and depth of spawning were based primarily on the closely related Kamchatka flounder, *A. evermanni*. More recent genetic and food-habit studies have documented differences between arrowtooth flounder and Kamchatka flounder in the Bering Sea (e.g. Ranck et al., 1986; Yang and Livingston, 1986; Yang, 1988), but none have examined their reproduction. Unpublished arrowtooth flounder size-at-maturity estimates have been made from data collected off Oregon.[3] This study was designed to provide new estimates of size at maturity and a time series of reproductive development and spawning of arrowtooth flounder off Washington.

Methods

Maturity data were collected from three sources: 1) Washington commercial trawl landings ("market"-sized fish) sampled biweekly from July 1991 to July 1992 at shoreside processing plants; 2) fish normally discarded at sea because of their small size, brought in by fishermen

[1] Wilderbuer, T. K., and E. S. Brown. 1992. Flatfish. *In* Stock assessment and fishery evaluation report for the 1993 Gulf of Alaska groundfish fishery, Section 3. N. Pac. Fish. Manage. Counc., Anchorage, AK, 25 p.

[2] Pacific Fishery Management Council. 1993. Status of the Pacific coast groundfish fishery through 1993 and recommended biological catches for 1994: stock assessment and fishery evaluation. (Document prepared for the Council and its advisory entities.) Pac. Fish. Manage. Counc., 2000 SW First Ave., Ste. 420, Portland, OR 97201.

[3] Hosie, M. J., and W. H. Barss. 1977. Age and length at maturity of arrowtooth flounder, *Atheresthes stomias*, in Oregon waters. Oregon Dep. Fish Wildl. unpubl. manuscr., 13 p.

upon request (discards); and 3) survey samples collected at sea from September to November 1992 on the National Marine Fisheries Service (NMFS) triennial bottom trawl and slope surveys off Washington State and Vancouver Island, British Columbia (Table 1). All of the commercial trawlers sampled used larger codend mesh than did the trawl survey (11.4 cm and 8.9 cm, respectively) and reported arrowtooth flounder catch from grounds off northwest Washington (Fig. 1). Commercial trawlers combine catches from different tows so trawl landings and landed discards were sampled on a per trip basis. No samples were obtained in January or February 1992. Up to 100 fish were selected at random for each sample. If fewer than 100 fish were present, all fish were sampled. Each fish was measured to the nearest centimeter fork length (FL), sexed, and assigned a maturity stage based on the macroscopic appearance of the gonads (Table 2). A gonosomatic index (GSI), calculated as ovary weight divided by somatic weight (body weight with ovaries removed and stomach empty), was used to track seasonal changes in ovarian condition. In each sample, the first 10 females encountered at each macroscopic maturity stage were sampled for GSI. Whole ovaries were removed and weighed to the nearest gram, and somatic weight was recorded to the nearest gram. Average monthly GSI's were calculated by maturity stage.

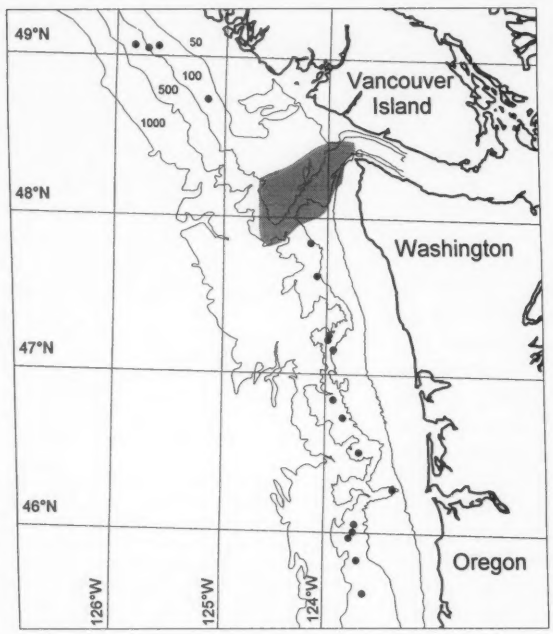

Figure 1

Reported arrowtooth flounder, *Atheresthes stomias*, catch area for sampled commercial bottom trawl trips, and locations of survey tows sampled for maturity, with fathom contours (1 fm=1.83 m).

Fish at any maturity stage other than "immature" were defined as mature. Length at maturity was estimated for each sex by calculating the fraction mature in each 1-cm interval and by fitting a logistic model

$$P_L = \frac{1}{1+e^{(a+bL)}},$$

where P_L = fraction mature at length L, and a and b are constants (Gunderson et al., 1980). The predicted size at which 50% of the fish are mature is

$$L_{50\%} = \frac{-a}{b}.$$

Confidence intervals for $L_{50\%}$ were calculated by using the delta method (Seber, 1982). To see if time of collection affected estimates of $L_{50\%}$, logistic equations were fit to the female length-maturity data that were grouped into the following periods for 1991 and 1992 separately: September–December, January–April, and May–August, corresponding to the prespawning, spawning, and postspawning seasons as determined below.

Ovarian tissue samples were collected from five tows on board the commercial trawler FV *Larkin* in late December 1991, during normal trawl operations off northwest Washington, to compare microscopic and macroscopic staging and provide additional information on arrowtooth flounder reproduction. Immediately after the net was retrieved, arrowtooth flounder were sorted by the crew into discard and market categories and were held alive until they could be sampled. Discard and market categories were maintained for maturity sampling but combined for histology. Length, sex, and maturity stage were recorded, and for the first 10 females encountered at each maturity stage an approximately 3-mm section of ovarian tissue was cut from the anterior third of the blind-side (left) ovary, placed in a tissue cassette, and fixed in Davidson's fixative (Mahoney, 1973). Tissue samples cut from the anterior third of the ovary were assumed to be representative of the arrowtooth flounder ovary as a whole. Weights for GSI were not taken owing to rough weather.

After fixation, tissues were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. Tissue slides were examined under a compound microscope and measurements were taken with a calibrated ocular micrometer. A classification scheme of five stages of oocyte development, two stages of atresia, and a final stage of postovulatory follicles was adopted (Table 3), with terminology proposed by

Table 1

Sources of reproductive data on arrowtooth flounder, *Atheresthes stomias*, with numbers of fish sampled for maturity, and numbers of females sampled for GSI and histology. Samples were collected per trip obtained shoreside or collected per tow obtained at sea; average minimum depth[1] is the average of the minimum depths for tows with arrowtooth flounder from sampled trips and the minimum depth fished for sampled tows. Sampling includes mean fork length (cm) and standard deviation (SD) of fish sampled for maturity with probability *P* for test of difference between means (1-tailed Student's *t*-test, $\alpha = 0.05$).

Sample category	Year	Month	Number of samples		Average minimum depth (m)	Number of fish				Males		Females		
						Maturity								
			Trips	Tows		Males	Females	GSI	Histology[2]	Mean length	SD	Mean length (cm)	SD	*P*
Market	1991	July	2		140	1	199	43		53.0	—	62.1	4.8	—
		August	1		141	1	99	15		45.0	—	60.8	6.0	—
		September	3		196	25	275	70		44.8	2.1	62.1	9.2	0.001
		October	2		240	27	173	56		43.3	4.2	57.8	9.4	0.001
		November	1		265	17	83	25		39.7	1.9	54.2	10.1	0.001
		December	2	4	431	37	330	59	105	35.4	3.9	45.4	8.6	0.001
	1992	March	1		283	15	85	20		37.9	4.9	45.7	9.2	0.001
		April	2		304	46	154	26		43.6	3.1	56.1	10.1	0.001
		May	2		279	32	168	25		44.6	2.8	63.9	9.1	0.001
		June	2		242	24	176	44		45.6	2.2	62.8	8.1	0.001
		July	2		148	91	109	40		44.5	3.0	57.3	7.4	0.001
Discard	1991	December		2	393	74	26		6	29.6	2.4	28.8	1.3	0.050
	1992	May	1		214	10	6	6		37.6	6.9	32.5	6.5	0.083
		June	1		232	24	45			33.2	2.8	31.0	3.5	0.004
Survey[3]	1992	September		4	163	58	63	46		38.9	7.6	46.5	14.6	0.001
		October		7	335	49	85	20		33.6	7.1	40.8	12.5	0.001
		November		7	403	37	59	22		31.0	4.7	41.5	13.9	0.001

[1] Minimum depth is defined as the shallowest depth recorded for either start or end position of a tow.
[2] Histology samples collected at sea.
[3] GSI samples collected at sea, from 4 tows in September, 1 tow in October, and 1 tow in November.

Table 2

Maturity code definitions for arrowtooth flounder, *Atheresthes stomias*, based on the macroscopic appearance of the gonads.

Sex	Stage	Description
Female	Immature	Ovaries are small, pink to red in color, and gelatinous. Little or no visible internal ovarian tissue structure and no oocytes are visible.
	Mature	
	Developing	Ovaries are enlarging. Visible oocytes are white, opaque, and granular.
	Gravid	Ovaries are yellowish and mottled; some oocytes are translucent.
	Ripe/Running	Ripe and running. All oocytes are translucent and may be extruded with light pressure.
	Spent/Resting	Spent ovaries are flaccid, bloodshot, and red to purple. Resting ovary wall looks toughened, pink to beige.
Male	Immature	Testes small and thread-like, brown to pink. No folding or mottled coloration.
	Mature	Testes enlarged, folded, brown to white. Sperm may be visible in cross section or flows with external pressure.

Yamamoto (1956) and stage definitions used by Yamamoto (1956), Wallace and Selman (1981), Hunter and Macewicz (1985), and West (1990). The presence of α and β atretic oocytes was noted, although no attempt was made to differentiate α atretic oocytes by developmental stage. Postovulatory fol-

Table 3

Oocyte developmental stages based on histological criteria.

Stage	Description
Chromatin nucleolar	The oocyte is surrounded by a few squamous follicle cells, and scant cytoplasm surrounds a large nucleus containing a single, large basophilic nucleolus.
Perinucleolar	The oocyte grows, the nucleus increases in size and multiple basophilic nucleoli appear. These arrange themselves towards the periphery of the nucleus; a single dark dot or "Balbiani body" may be visible in the outer surface of the cytoplasm.
Cortical alveoli formation	Spherical vesicles appear in the cytoplasm. Zona radiata present.
Vitellogenic (yolk)	Oocytes are greatly increased in size and ooplasm is filled with yolk globules. Nucleus may be toward the periphery; there is a rapid thickening of the zona radiata.
Hydrated	Yolk globules fuse to form a continuous mass of fluid and the nucleus is not apparent. Postovulatory follicle may be visible.
α atresia	Oocyte is resorbed. Oocyte characterized by an irregular shape and granular, dark basophilic staining. When resorption is complete all that remains is the follicle.
β atresia	Follicle degenerates and is resorbed. Follicle compact, composed of several granulosa cells surounded by a thecal and blood vessel layer. Yellow-brown pigment may be present.
Postovulatory follicle	Collapsing follicle is irregularly shaped; cell layers form loose folds or loops. Degenerating follicle shows fewer loops and is reduced in size.

licles (POF) were identified except in later stages of degeneration when they could not be differentiated from β atresia (Hunter and Macewicz, 1985). Ovaries were staged on the basis of the most developmentally advanced oocyte observed. Proportions of oocyte stages, atretic structures, and POF's were calculated from counts of structures passing under a line horizontally bisecting the tissue slide. Because oocytes at different stages of development differ greatly in size, counts may be biased against smaller, less-developed stages. Average oocyte diameter was determined from the first 10 oocytes encountered at the most advanced developmental stage and sectioned through the nucleus, except hydrated oocytes which were measured as encountered.

Washington commercial bottom trawl logbook data from July 1991 to July 1992 were examined for trends in fishing depth and arrowtooth flounder catch rates. Information on the target fish and estimates of the amount of fish discarded at sea were unavailable. Catch rates were calculated from the set of 14,559 bottom trawl tows in which reported hours fished were greater than 0. Depth of each tow was defined as the shallowest (minimum) depth recorded for either start or end position. Catch rate or catch per unit of effort (CPUE) was computed as the average of the tow-by-tow arrowtooth flounder catch divided by hours fished; catch data were not adjusted to reflect actual fish ticket landings.

Results

Arrowtooth flounder larger than 53 cm FL were all female and the size ranges in each of the sample categories differed (Fig. 2). Females accounted for 85% (by number) of market, 42% of discard, and 67% of survey samples. Males were not well represented and did not show grossly apparent developmental changes over time; therefore, males were categorized only as mature or immature and were not considered further. However, males appeared most developed in July when the testes were largest, brown to whitish and folded. No spawning males were seen.

The average length of males was less than that of females in every month that market and survey samples were taken (Table 1; 1-tailed Student's *t*-test, α=0.05). Fish size (Table 1) and catch rates (Table 4) were highest in spring and summer. In winter, average length of market-sample fish decreased by approximately 9.0 cm for males and 16.0 cm for females. CPUE peaked (>200 kg/hr) in spring and fall at depths of 183–365 m and decreased in winter, while above 183 m CPUE was highest (about 50–150 kg/hr) in July–August but dropped to at or near 0.0 kg/hr in November–March. There was no apparent seasonal trend in CPUE at depths >366 m.

The proportion of mature females at each macroscopic maturity stage varied seasonally (Fig. 3). Because spring discards included fish that were the

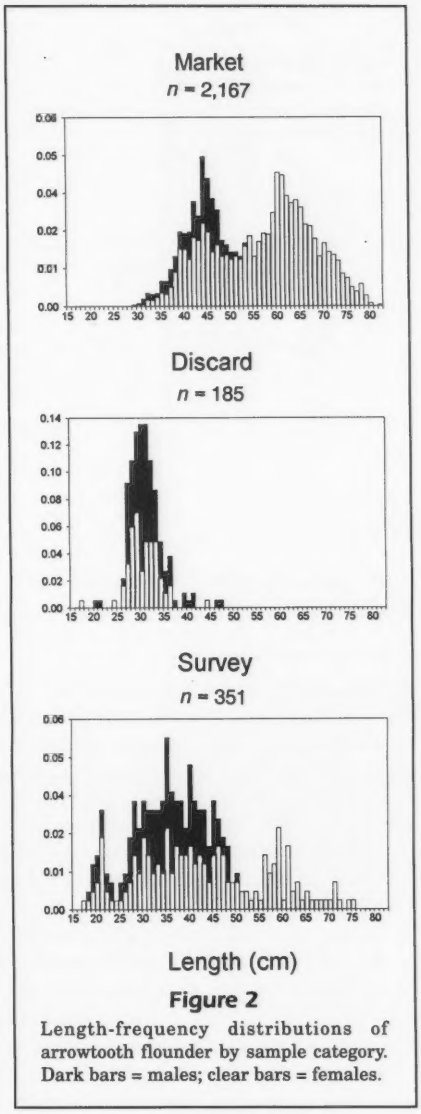

Figure 2

Length-frequency distributions of arrowtooth flounder by sample category. Dark bars = males; clear bars = females.

same size as fish in winter market samples, discard and market samples were pooled by common month and by keeping years separate. Throughout the year, samples almost always included large spent/resting females that did not show signs of ovarian recrudescence. Gravid females first appeared consistently in September 1991. The proportion of developing, gravid, and spent females stayed relatively constant through November. In December the first ripe/running fish and a substantial increase in the proportion of spent females were seen. The next available sample was March 1992 when all the mature females were in the spent/resting stage. Developing females reappeared the following May, and their proportion increased into the fall. In 1992, the first gravid and ripe/running fish were seen in November.

Length at 50% maturity calculated from survey data was 28.0 cm for males and 36.8 cm for females (Table 5). Estimates of $L_{50\%}$ from pooled market and discard ("commercial") data were lower for males and higher for females than estimates from survey data, although confidence intervals for $L_{50\%}$ overlapped. For females, seasonal estimates of $L_{50\%}$ varied widely. The greatest $L_{50\%}$ (>41 cm) was seen before spawning (May–August) and the lowest (<37 cm) during spawning (September–December). Parameters for the logistic function were compared with a likelihood-ratio test (Kimura, 1980). Estimates from commercial data were significantly different from survey estimates for both females (χ^2=145.490, P<<0.001; Fig. 4) and males (χ^2=79.383, P<<0.001). In a comparison of years, logistic curves fit to September–December 1991 (commercial) and September–November 1992 (survey) data were significantly different (likelihood-ratio test, χ^2=143.257, P<<0.001) although again confidence intervals for $L_{50\%}$ overlapped.

Ovarian tissue samples were analyzed histologically from 111 female arrowtooth flounder collected late December 1991 during spawning. Each of the five macroscopic maturity stages was represented and no two macroscopic stages showed the same frequency distribution of oocyte types (Fig. 5). Chromatin nucleolar, perinucleolar, and atretic oocytes were present in all the sampled ovaries. In ovaries of immature fish, none of the oocytes had progressed beyond the perinucleolar stage. Vitellogenic or yolked oocytes were prevalent in developing and gravid stage ovaries, and hydrated oocytes were seen frequently in gravid and ripe/running stage ovaries. Oocytes with cortical alveoli were most frequently seen in spent/resting ovaries.

Atresia was more prevalent in all the ovarian stages of spent/resting fish than in immature fish (Table 6). The percent occurrence of α atretic oocytes was lowest in ovaries from developing fish and highest in ovaries from spent/resting fish. Beta atresia was most common in spent/resting ovaries but also occurred in developing, gravid, and immature ovaries. All the immature and 43.8% of the spent/resting females had perinucleolar stage ovaries. Postovulatory follicles (POF) were present in ovaries from all macroscopic stages except immature. POF were most frequently seen in ripe/running ovaries and were common in gravid and spent/resting ovaries; whereas 7 of 29 developing females examined for histology had ovaries with POF. Postovulatory follicles were also present in 7 of 21 spent/resting

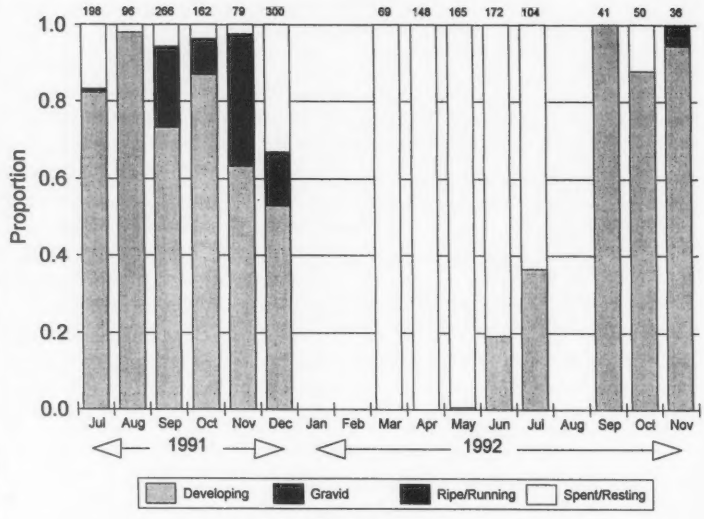

Figure 3

Proportions of mature female arrowtooth flounder at each macroscopic maturity stage by month. Number of females listed at top.

Table 4

Washington commercial bottom trawl catch rates (kg/hr) of arrowtooth flounder, *Atheresthes stomias*, by depth interval based on the minimum depth recorded for each tow. n = number of tows.

		1–182 m		183–365 m		366–547 m		547+ m	
Year	Month	n	kg/hr	n	kg/hr	n	kg/hr	n	kg/hr
1991	July	1,644	155.7	176	184.0	31	44.6	9	134.5
	August	1,391	54.5	179	130.6	21	3.0	5	0.0
	September	1,362	14.5	187	157.6	54	27.7	7	0.0
	October	860	11.8	274	231.7	56	64.3	4	0.0
	November	210	0.0	95	20.3	37	22.7	3	0.0
	December	187	0.0	126	8.9	82	35.8	7	0.0
1992	January	221	0.1	120	14.9	82	8.9	18	11.0
	February	596	0.0	240	10.7	61	12.2	37	0.4
	March	883	0.3	201	19.5	178	39.8	85	8.9
	April	462	7.4	155	53.9	110	68.5	31	1.7
	May	829	16.3	232	228.2	63	71.0	40	0.0
	June	1,131	19.0	174	344.1	38	16.4	90	0.5
	July	1,246	53.4	134	50.8	13	1.7	82	1.5

females with perinucleolar-stage ovaries. One 41.0-cm gravid female had no POF and 6.3% of its oocytes were hydrated. All other gravid females had POF.

Overall mean oocyte diameters (μm) and standard deviations were as follows: perinucleolar 79.6 ± 34.9 (n=420); cortical alveoli 185.4 ± 23.4 (n=220); vitellogenic 722.9 ± 73.9 (n=290); and hydrated 940.6 ± 206.8 (n=170) (Fig. 6). Chromatin nucleolar stage oocytes were not measured because they never represented the most advanced stage present in an ovary. Mean diameter of perinucleolar oocytes in spent/resting females was about 10 μm greater than that in the immature females (Student's t-test, P<0.003). Some of the variance in hydrated oocyte size

Table 5

Parameter estimates for the logistic model[1] of proportion mature at length (cm), length at 50% mature, and 95% confidence intervals, for arrowtooth flounder, *Atheresthes stomias* from 1991–92 Washington commercial (pooled market and discard), 1992 Washington survey, and 1972–75 Oregon data (See Footnote 3 in the text). For females, results are also given for Washington commercial data grouped by months in relation to the spawning season.

Sex and stratum	a	b	R^2	$L_{50\%}$ (cm)	95% CI for $L_{50\%}$	n
Males						
Washington-survey	24.990	–0.893	0.968	28.0	27.6–28.4	144
Washington-commercial	33.264	–1.197	0.968	27.8	27.5–28.1	424
Oregon	17.397	–0.615	0.971	28.3	27.8–28.8	218
Females						
Washington-survey	19.891	–0.540	0.971	36.8	36.3–37.3	207
Washington-commercial	20.874	–0.559	0.984	37.4	37.0–37.7	1,928
Oregon	15.056	–0.352	0.989	42.8	42.4–43.2	1,628
Females[2]						
Jul–Aug 1991 (before)	13.050	–0.281	0.390	46.5	43.2–49.7	298
Sep–Dec 1991 (during)	14.800	–0.410	0.945	36.1	35.5–36.7	887
Mar–Apr 1992 (after)	61.111	–1.568	0.991	39.0	38.8–39.1	239
May–Jul 1992 (before)	18.194	–0.438	0.928	41.6	40.6–42.6	504

[1] $P_L = 1/(1+e^{(a+bL)})$.

[2] No samples obtained in January–February 1992.

can be attributed to distortion of hydrated oocytes that occurs during histological processing.

Gonosomatic indices (GSI) for immature and spent/resting fish remained fairly constant across seasons, whereas GSI for developing or gravid fish varied considerably over time, reflecting ovarian recrudescence (Table 7). Individual GSI for immature fish ranged from 0.0017 to 0.0154 (average 0.0055), and individual spent/resting GSI ranged from 0.0037 to 0.0259 (average 0.0135). GSI for ripe/running fish (n=4; $\bar{L}$=47.8) was not calculated because loss of eggs in handling precluded accurate gonad weights. From histological analysis, hydrated oocytes and POF were found in "developing" females, indicating some misclassification of spawning females. Because of suspected misclassification, developing and gravid GSI data were pooled by month. GSI for developing or gravid fish showed a rapid increase from about 0.03 in August 1991 and 0.02 in July 1992 to 0.06 in September of both years; the highest annual mean GSI occurred in December 1991 and November 1992. The highest individual GSI was 0.2201 in November 1992 for a 38-cm gravid female.

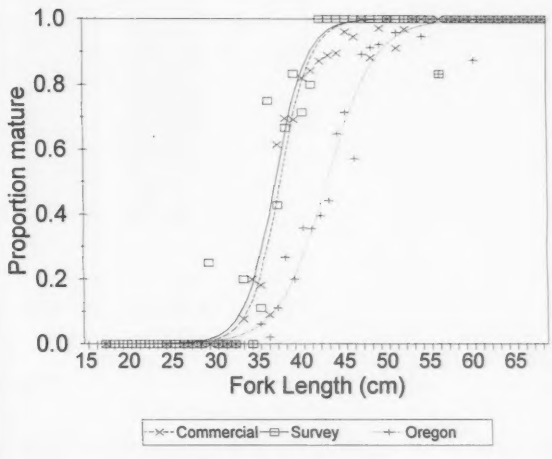

Figure 4

Maturity distributions and logistic curves for female arrowtooth flounder calculated from pooled 1991–92 market and discard (commercial) data, 1992 survey data, and 1973–75 data from Oregon (see Footnote 3 in the text).

Discussion

The progression of gonad maturity stages, increase in GSI, and the appearance of gravid females show that arrowtooth flounder spawn during fall–winter off Washington. Spawning begins as early as September, extends at least through December, and is completed by March. This coincides with the spawning season for *Atheresthes evermanni* in the western

Table 6

Percent occurrence of oocytes in each oocyte developmental stage in arrowtooth flounder, *Atheresthes stomias*, by macroscopic and microscopic maturity stages. Percent occurrences of atresia and postovulatory follicles (POF) among all oocyte structures also included. Microscopic stage reflects the most advanced oocyte type present. n = number of females, L = mean FL in cm; oocyte-stage 1 = chromatin nucleolar; stage 2 = perinucleolar; stage 3 = cortical alveoli; stage 4 = vitellogenic; stage 5 = hydrated; and POF = postovulatory follicle. Samples were collected late December 1991.

Macroscopic stage	Microscopic stage	n	$\bar{L}$ (cm)	Oocyte developmental stage					Atresia		POF
				1	2	3	4	5	α	β	
Immature	Perinucleolar	17	35.7	19.0	81.0	0.0	0.0	0.0	11.5	1.3	0.0
Developing	Vitellogenic	26	43.0	2.4	20.7	0.1	76.8	0.0	3.6	0.9	0.5
Developing	Hydrated	3	39.7	1.0	20.6	0.0	77.0	1.4	6.6	0.9	0.9
Gravid	Hydrated	15	40.4	3.1	25.5	0.2	47.0	24.1	7.2	0.9	7.1
Ripe/Running	Hydrated	2	59.0	3.3	39.6	0.0	1.1	56.0	7.2	0.0	26.8
Spent/Resting	Perinucleolar	21	48.2	15.8	84.2	0.0	0.0	0.0	21.7	7.3	5.4
Spent/Resting	Cortical alveoli	26	61.8	14.5	76.1	9.4	0.0	0.0	20.1	10.2	2.9
Spent/Resting	Hydrated	1	42.0	6.3	34.4	0.0	34.4	25.0	16.7	0.0	7.1

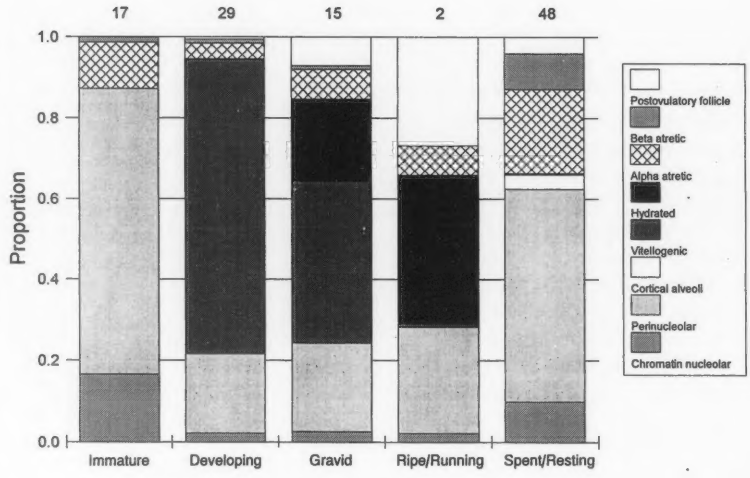

Figure 5

Proportion of arrowtooth flounder oocytes assigned by histological criteria to ovarian developmental stages according to macroscopic maturity stage of the whole ovary. Samples were collected late December 1991. Number of females is listed at top.

Bering Sea reported by Pertseva-Ostroumova (1960). Hirschberger and Smith (1983) found some spawning arrowtooth flounder during spring and summer months in the Gulf of Alaska, but their survey data were insufficient to define the spawning season with any certainty. The appearance of translucent oocytes is usually taken as an indication that spawning is imminent (hours or days) (West, 1990), although there are no laboratory data to indicate the speed at which arrowtooth flounder oocytes ripen and are ovulated. Gravid females were first seen in September 1991 coincident with an increase in GSI. A similar increase in September GSI was seen in 1992, but the first gravid females were observed in November, suggesting that the start of arrowtooth flounder spawning may vary from year to year.

Histological results show arrowtooth flounder are batch spawners, where one female spawns repeat-

Table 7

Arrowtooth flounder, *Atheresthes stomias*, monthly mean fork length ($\bar{L}$, in cm) and mean gonosomatic index (GSI) and standard deviation (SD) for females selectively sampled for GSI by macroscopic maturity stage. July 1991–July 1992 samples were collected shoreside; September–November 1992 samples were collected at sea.

		Immature				Developing/Gravid				Spent/Resting			
Year	Month	*n*	$\bar{L}$	GSI	SD	*n*	$\bar{L}$	GSI	SD	*n*	$\bar{L}$	GSI	SD
1991	July	1	48.0	0.0056	—	22	63.3	0.0277	0.0096	20	61.1	0.0164	0.0021
	August	3	48.0	0.0080	0.0035	10	60.4	0.0290	0.0082	2	63.5	0.0120	0.0028
	September	9	42.8	0.0064	0.0020	46	65.1	0.0587	0.0249	15	53.9	0.0163	0.0047
	October	10	39.4	0.0051	0.0006	40	61.0	0.0738	0.0246	6	56.7	0.0120	0.0066
	November	3	41.7	0.0045	0.0003	20	55.7	0.0883	0.0223	2	54.5	0.0051	0.0003
	December	14	36.3	0.0048	0.0018	33	47.0	0.1160	0.0332	11	45.7	0.0070	0.0060
1992	March	10	35.9	0.0045	0.0017	0	—	—	—	10	49.5	0.0126	0.0015
	April	6	40.5	0.0051	0.0013	0	—	—	—	20	55.3	0.0114	0.0035
	May	9	36.6	0.0048	0.0030	1	67.0	0.0140	—	21	65.4	0.0135	0.0026
	June	4	44.5	0.0067	0.0009	20	61.9	0.0171	0.0042	20	64.4	0.0151	0.0030
	July	5	41.0	0.0071	0.0034	17	57.8	0.0215	0.0046	18	53.8	0.0145	0.0031
	September	18	29.2	0.0063	0.0042	28	55.6	0.0615	0.0292	0	—	—	—
	October	10	35.9	0.0049	0.0017	10	46.0	0.0567	0.0303	0	—	—	—
	November	10	30.0	0.0056	0.0041	11	46.4	0.1004	0.0487	0	—	—	—

edly over a protracted spawning season, as are Pacific halibut, *Hippoglossus stenolepis* (St-Pierre, 1984), and Dover sole, *Microstomus pacificus* (Hunter et al., 1992). Size frequencies of oocyte stages (Fig. 6) show distinct populations of oocytes that indicate a group-synchronous pattern of development. Postovulatory follicles were found in "developing" females, those with no visible translucent oocytes, evidence that these fish had recently spawned and that macroscopic examination of ovaries could not separate all spawning from nonspawning fish. In September 1991, early in the spawning season, gravid mean GSI was significantly greater than developing GSI (Student's *t*-test, $P<<0.001$), but by November and December there was essentially no difference between mean GSI for developing and gravid females (Student's *t*-test, November $P<0.105$, December $P<0.841$); the highest mean GSI was observed in December. Under the macroscopic maturity definitions used, females progress from immature to the developing stage, then cycle between "developing" and "gravid" as successive batches of oocytes ripen and are ovulated. The ripe/running stage corresponds then only to the last and perhaps largest batch of oocytes, suggesting that by December spawning was near completion for some females.

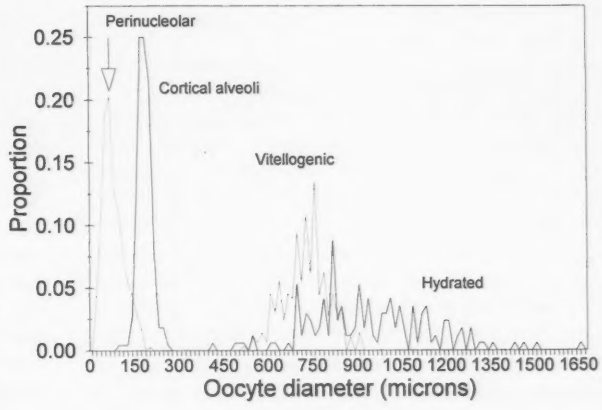

Figure 6

Size-frequency distributions of arrowtooth flounder oocyte developmental stages from ovaries collected late December 1991. Proportions were calculated separately for each oocyte type.

Spent/resting females were seen year-round, evidence that adult arrowtooth flounder may not spawn every year.

Of particular concern is whether small, resting mature fish were misclassified as immature, and vice versa, since errors will bias estimates of size at maturity. Immature male arrowtooth flounder were difficult to identify and errors undoubtedly occurred,

but there was not enough auxiliary information to quantify them. Macroscopically immature females examined for histology did not show signs of recent spawning, and all were microscopically staged as perinucleolar. Out of 48 spent/resting females examined for histology, 43.8% also had perinucleolar ovaries. Seven of these had POF, direct evidence of prior spawning. Of the remaining 14, atretic structures were more than twice as common as in immature females. Hunter et al. (1992) used atresia to distinguish immature from uncertain maturity in Dover sole ovaries defined as inactive but concluded that microscopic examination of oocytes in histological sections may not identify all mature, postspawning females. Relatively high rates of atresia may indicate that fish have finished spawning (Wallace and Selman, 1981), but this alone is insufficient to separate mature from immature fish because atresia can be brought on by stresses not necessarily associated with spawning, such as starvation, pollution, or other environmental conditions (Wallace and Selman, 1981; Hunter and Macewicz, 1985; Hunter et al., 1992). Because histological criteria could not differentiate all mature from immature females and because other microscopic evidence of prior spawning such as ovarian wall thickness (Burton and Idler, 1984) was not available, the degree of misclassification of mature vs. immature females staged macroscopically could not be determined.

Histological processing is known to cause shrinkage of oocytes (West, 1990); therefore oocyte diameters determined from processed tissue sections should be considered an index rather than an absolute measurement of oocyte size. For arrowtooth flounder, Pertseva-Ostroumova (1960) reported whole egg diameters are about 2.5–3.5 mm. Matarese et al. (1989) lists *A. stomias* egg diameter as approximately 3 mm, three times the mean diameter of ripe oocytes determined in this study.

Seasonal bathymetric migrations are a familiar pattern in flatfish. Dover sole, *Microstomus pacificus*, petrale sole, *Eopsetta jordani*, and English sole, *Pleuronectes vetulus*, migrate seasonally across depths (Alverson et al., 1964), and Dover sole tend to reside at deeper depths at older ages (Hunter et al., 1990). Kabata and Forrester (1974) sampled arrowtooth flounder off Vancouver Island in May–June 1968 and found increasing length with depth, and a drop in abundance below 420 m (230 fm) consistent with these results. Trends in arrowtooth flounder catch rates by depth and season (Table 4) indicate arrowtooth flounder move offshore in winter. Arrowtooth flounder were common in shallow water (<183 m) in summer, when the average size of landed fish was large. In winter, smaller numbers and sizes of arrowtooth flounder were caught in deeper water; whereas several hundred tows were reported in shallow water with virtually no arrowtooth flounder. Large, mature, presumably spawning arrowtooth flounder may have moved out of the range of the trawl fishery, possibly to deeper water or north into Canadian waters. However, targeted arrowtooth flounder trips are rare and independent estimates of the amounts of arrowtooth flounder discarded from trawl catches are high, from nearly 76% off Oregon and Washington (Barss and Demory, 1985) to over 80% in the Gulf of Alaska.[1] Large volumes of discards and catch unreported in logbooks may obscure trends in distribution; they certainly result in underestimates of arrowtooth flounder CPUE. Hosie (1976) states that arrowtooth flounder spawn off central Oregon from December through March at about 200 fm. Hirschberger and Smith (1983) reported spawning arrowtooth flounder at depths of over 350 m (191 fm) in the Gulf of Alaska. The full extent of the spawning depth range inhabited by arrowtooth flounder has not been determined, but in this study in 1991 gravid females were found in commercial tows out to 512 m (280 fm) and ripe/running females at 475 m (260 fm). In 1992 gravid and ripe/running females were found at 399 m (218 fm). Since these results also suggest that in winter the bulk of the population was in water as deep or deeper than 366 m, it is likely that the majority of arrowtooth flounder off Washington spawn at depths exceeding 366 m (200 fm).

To examine trends in length at maturity over time, I fitted logistic curves to Oregon trawl survey data from the 1970's[3] to compare with results from Washington (Table 5). The Oregon survey covered the area from Newport, Oregon, south to Cape Blanco and included FL and maturity for 218 male and 1,628 female arrowtooth flounder. Macroscopic criteria used to distinguish mature from immature fish were identical in both studies. Washington maturity samples were collected in all months except January and February. All months except July, August, and November were represented in the Oregon data. Arrowtooth flounder in the present study matured at a smaller size than those collected off Oregon, and likelihood-ratio tests (Kimura, 1980) showed sample nonlinear regressions for Oregon were significantly different when compared with both Washington survey data (male χ^2=74.555, P<<0.001; female χ^2=137.922, P<<0.001) and commercial data (male χ^2=80.539, P<<0.001; female χ^2=147.920, P<<0.001). Distribution of female maturities across lengths (Fig. 4) suggests that female arrowtooth flounder are maturing at a smaller size than they were off Oregon in the early 1970's, or that there are latitudinal

differences in size at maturity. However, differences between results for Oregon and the present study could be explained by differences in sampling distributions across months, or by different interpretations of maturity codes by different observers; therfore they may not represent a biological change.

Size-selectivity and areal or bathymetric sampling biases are critical to estimates of $L_{50\%}$ (Welch and Foucher, 1988; Trippel and Harvey, 1991). Predicted length at maturity may be biased if fish are size-segregated by area or depth (e.g. if immature fish do not migrate to spawning depths while targeted commercial trawl fisheries typically operate where large fish are most likely to be found in quantity, i.e. the spawning grounds). Smaller arrowtooth flounder were not well represented in commercial landings. Size selectivity in commercial fisheries occurs either through net selection (a lesser problem in the trawl survey) or through size-selective targeting and discarding. Net avoidance by larger fish may also be a factor. In the extreme case of male arrowtooth flounder, estimated $L_{50\%}$ was below the size range of fish in market samples though well within the size range of fish in commercial discards and survey catches. Because the trawl survey sampled the widest size range of arrowtooth flounder over a fairly large area and depth range prior to peak spawning in 1992, size-at-maturity estimates from the trawl survey represent the best available.

Hunter et al. (1992) estimated size at maturity for female Dover sole and found samples taken during the spawning season yielded higher estimates of $L_{50\%}$ than did samples taken before spawning. They attributed this to the presence of postspawning females with "highly regressed" ovaries that were histologically indistinguishable from immature females, and they concluded that estimates of length or age at first maturity should always be conducted prior to the onset of spawning, when such females are rare. I found the opposite seasonal pattern in length at maturity for female arrowtooth flounder; lowest $L_{50\%}$ was estimated from commercial samples collected in the fall during spawning, and highest $L_{50\%}$ was estimated from months preceding spawning. Market samples collected in summer (before spawning) tended to underrepresent smaller arrowtooth flounder and yielded extremely high estimates of female length at maturity. In the case where sampling is limited to commercial trawl fisheries, it may be preferable to pool year-round sampling data to generate estimates of $L_{50\%}$ if fish are moving in and out of range of the trawl fleet, rather than to attempt to narrow the sampling window to just prior to spawning as suggested by Hunter et al. (1992). This involves the explicit trade-off of some assumed increase in the misclassification of small fish with the significant bias caused by a seasonal inability to obtain representative samples of the entire size or bathymetric range of a population. Perhaps coincidentally, female length-at-maturity curves generated from the year-round commercial data and the survey data were strikingly similar (Fig. 4), although statistically the curves were different.

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Marine turtle populations on the east-central coast of Florida: results of tagging studies at Cape Canaveral, Florida, 1986–1991*

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Abstract.—Marine turtle tagging records were collected off east-central Florida by a shrimp trawler from May 1986 to December 1991. The data were analyzed to determine species composition, size distribution, seasonal occurrence, movements, morphometrics, and growth of 928 incidentally captured turtles. Loggerhead turtles, *Caretta caretta*, were the most frequently captured species (83% of total catch), while Kemp's ridley, *Lepidochelys kempi*, and green turtles, *Chelonia mydas*, were caught less frequently (12% and 4%, respectively). The loggerhead turtle population consisted of a seasonably variable aggregation of subadult and adult turtles. The Kemp's ridley and green turtle populations were composed of subadult turtles and were captured primarily during winter months. Kemp's ridley and loggerhead turtles appeared to exhibit a seasonal north-south migrational pattern along the Atlantic coast. Regression equations were developed for the morphometric relationships of each species. Average yearly growth rates and estimates for the von Bertalanffy growth interval equation were calculated for loggerhead and Kemp's ridley turtles. These results indicate that the coastal waters of the Cape Canaveral area provide an important developmental habitat for the three species of marine turtle.

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The marine turtle life history is a dynamic progression of stages which includes oceanic dispersal of the offspring and utilization of a series of distinct developmental habitats (Carr, 1980; Hendrickson, 1980). The early in-water stages of marine turtle development have not been as extensively studied as the reproductive stage of females. Information concerning the early life histories of threatened and endangered marine turtles is critical in formulating conservation and recovery strategies as mandated by the Endangered Species Act of 1973 and subsequent amendments.

Inaccessibility of immature turtles in the open ocean is the major factor contributing to the lack of information on the early stages of development. Other than possible current-mediated dispersal scenarios (Carr, 1986; Collard and Ogren, 1990), little is known about the pelagic stage of marine turtle development. However, information concerning the populations of immature turtles foraging in the coastal waters of eastern Florida has been accumulating as a result of data collected through commercial fisheries (fishery-dependent) and fishery-independent activities.

Fishery-independent capture and tagging efforts have characterized the populations of loggerhead turtles, *Caretta caretta*, and green turtles, *Chelonia mydas*, foraging in the northern part of the Indian River lagoon system (Ehrhart and Yoder, 1978; Mendonça, 1981, 1983; Mendonça and Ehrhart, 1982; Ehrhart, 1983). All green turtles collected in the lagoonal habitat were immature, as were almost all of the loggerhead turtles. Aggregations of marine turtles in the Port Canaveral ship channel were first reported in 1978, when two trawlers caught unprecedented numbers of loggerhead turtles while searching for a concentration of shrimp (Carr et al., 1980). This prompted the National Marine Fisheries Service (NMFS) to conduct trawl surveys of the ship channel from 1978 to 1984 (Butler et al., 1987; Henwood, 1987a; Henwood and Ogren, 1987). The surveys provided information on the seasonal occurrence and movement patterns of subadult and adult loggerhead turtles (Henwood, 1987a), as well as subadult Kemp's ridley, *Lepidochelys*

*Contribution MIA-92/93-94 of the Miami Laboratory, Miami, Florida.

kempi, and green turtles (Henwood and Ogren, 1987) captured in the vicinity of Cape Canaveral. In addition, the results of research conducted in response to incidental mortality of marine turtles due to dredging in the Port Canaveral ship channel were presented at the Cape Canaveral Sea Turtle Workshop (Witzell, 1987).

In 1986, the NMFS Panama City Laboratory initiated long-term studies of marine turtles found along the northwest (Cedar Keys) and east-central (Cape Canaveral) coasts of Florida (Schmid and Ogren, 1990). The Kemp's ridley turtle was the target species in both study areas. This paper presents the results of NMFS marine turtle studies conducted in nearshore waters of east-central Florida from 1986 to 1991. Information concerning marine turtle species composition, relative abundance, size frequency, seasonal occurrence, movements, morphometrics, and growth is provided.

Materials and methods

Data collection

A commercial shrimp-fishing vessel was contracted by NMFS, from May 1986 to December 1991, to measure, tag, and release marine turtles incidentally captured during trawling. Fishing effort and location were a function of the seasonal abundance of brown shrimp, *Penaeus aztecus*, and white shrimp, *Penaeus setiferus*. Trawling was conducted between St. Mary's Entrance, 30°43'N, and Sebastian Inlet, 27°52'N (Fig. 1), and was concentrated in the Cape Canaveral area, 28°30'N to 28°15'N, as defined by Henwood (1987a). Fishing effort did not extend beyond 24 km offshore and 25.6 m in depth. The majority of effort occurred less than 8 km offshore and 13.4 m in depth. Trawling gear consisted of four 12.2-m or 12.8-m nets (two on each side) for targeting brown shrimp, or two 24.4-m nets (one on each side) for targeting white shrimp.

Captured turtles were double tagged on the trailing edge of the fore flippers with #681 Inconel cattle ear tags. Tagging information for each turtle included: tag codes, species, date, location of capture, latitude and longitude, depth, gear type, standard straight-line carapace length (SSCL; nuchal notch to posterior end of postcentral), and straight-line carapace width. Carapace length and width were measured to the nearest 0.1 inch with forester's calipers and were converted to metric units for analysis. Kemp's ridley and green turtles were weighed with a 15-kg capacity spring scale. Notes on the condition of the turtle were recorded when the animal was injured or deformed (e.g. missing flipper, carapace wounds, etc.).

NMFS issued Sea Turtle Conservation Regulations on 29 June 1987 (Federal Register, 1987) that required vessels 25 feet (7.6 m) long or longer to use Turtle Excluder Devices (TED's) in the Cape Canaveral area beginning 1 October 1987. Subsequently, a NMFS permit was issued authorizing the contract vessel to conduct a TED testing program during fishing operations. The testing procedure consisted of towing a net(s) equipped with a Morrison soft TED on one side of the boat and a net(s) without a TED on the other side. Pounds of shrimp, marine turtle captures, and total catch (when possible) were recorded for the trawl types. Effort data were available for 1989–91, including trawl size and type, number of tows and total tow time, and number of days fished.

Data analysis

The terms "juvenile" and "subadult" used to describe the early stages of the marine turtle life history are

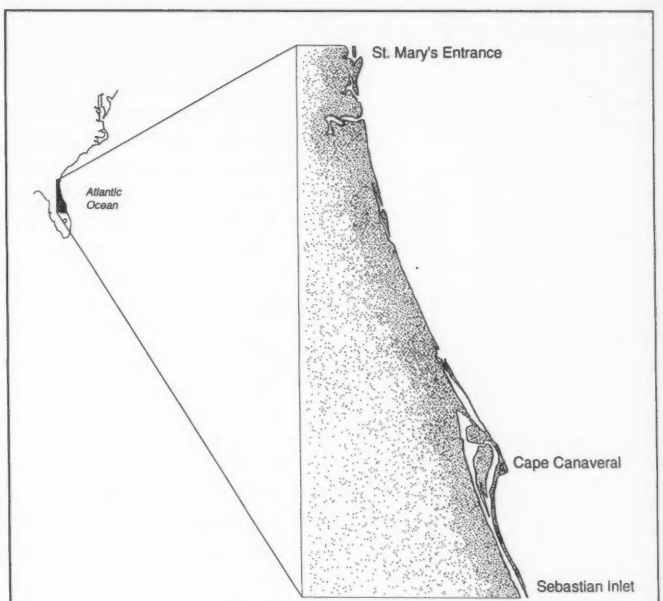

Figure 1
Sampling areas for marine turtles (*Caretta caretta*, *Lepidochelys kempi*, and *Chelonia mydas*) off the Atlantic coast of Florida from 1986 to 1991.

not well defined. In this study, the term "juvenile" has been reserved for immature turtles in the pelagic stage of development. A turtle is considered "subadult" when it has recruited to its respective coastal-benthic habitat and "adult" when sexually mature. Loggerhead turtles greater than 80-cm carapace length were considered adult, based on the length frequencies of Cape Canaveral nesting females (Carr, 1986, and references therein) and earlier investigations of Henwood (1987a). Kemp's ridley turtles greater than 60-cm carapace length were considered adult (Pritchard and Márquez, 1973).

Monthly trawling effort was calculated and standardized according to Henwood and Stuntz (1985) by using the formula

$$E = \left(\frac{Nets \cdot Length}{30.5}\right)\left(\frac{Min}{60}\right),$$

where E is the trawling effort in hours towed by a single 30.5-m headrope length net, *Nets* is the number of nets towed, *Length* is the headrope length (m) of a net, and *Min* is the number of minutes fished.

Capture records were analyzed to evaluate species composition within the study area, length-frequency distribution of each species, and patterns of seasonal distribution and movements. Linear regression analyses were performed for carapace width on length for loggerhead turtles. The morphometric data for loggerhead turtles were subdivided into a subadult group (<80 cm SSCL) and an adult group (≥80 cm SSCL) because the carapace dimensions of this species change as the animals mature (Henwood and Moulding, 1987). Carapace width was regressed on length, and weight regressed on length for Kemp's ridley and green turtles. Turtles with carapace wounds or deformities were not included in the analyses. Regression residuals for length-weight relationships were analyzed graphically to assess the appropriateness of the straight-line model (Sokal and Rohlf, 1981; Kleinbaum et al., 1988).

Curved carapace lengths (CCL) of stranded turtles were converted to straight-line carapace lengths (SCL) by using the following regression equations of Teas (1993):

$$SCL = -1.442 + (0.948 \times CCL)$$
for loggerhead turtles;

$$SCL = 0.013 + (0.945 \times CCL)$$
for Kemp's ridley turtles.

Total straight-line carapace lengths (*TSCL*) of loggerhead turtles were converted to standard straight-line carapace lengths (*SSCL*) with the regression equation of Henwood and Moulding (1987):

$$SSCL = (0.9964 \times TSCL) - 0.775.$$

Yearly growth rates were calculated from the formula

$$G = \left(\frac{\Delta Length}{Days}\right) 365$$

where G is the growth rate in cm/yr, $\Delta Length$ is the difference between the recapture length and the initial length, and *Days* is the number of days out. The von Bertalanffy growth interval equation was fitted to the recapture data with a nonlinear least-squares regression procedure (SAS, 1988). The von Bertalanffy growth interval equation (Fabens, 1965) for recapture is as follows:

$$CL_2 = a - (a - CL_1)e^{-kt},$$

where CL_2 is the carapace length at recapture, a is the asymptotic length, CL_1 is the length at first capture, K is the intrinsic growth rate, and t is the time in years between captures.

Results

Trawling effort

Monthly trawling effort varied from year to year (1989–91); however, monthly totals for all three years indicate that the majority of effort occurred from May to December (Table 1). This corresponds to the summer-fall fisheries for brown and white shrimp, the target species during this study. Monthly turtle capture rates were also variable, probably as a result of the combined seasonal fluctuations in trawling effort and turtle abundance. A structured sampling scheme with equal monthly effort would be required to make accurate calculations of monthly changes in turtle abundance. Loggerhead turtle catch per unit of effort (CPUE) ranged from 0.02 turtles/net hour in October 1989 to 1.09 turtles/net hour in August of 1991. Maximum CPUE of 0.25 turtles/net hour was obtained for Kemp's ridley turtles in May 1990 and 0.05 turtles/net hour was obtained for green turtles in January 1989 (Table 1).

Species composition

A total of 774 (83%) loggerhead, 113 (12%) Kemp's ridley, and 41 (4%) green turtles were captured, tagged, and released during the course of the study. A leatherback turtle, *Dermochelys coriacea*, was also

captured and tagged. Sixty tagged turtles (42 loggerhead, 15 Kemp's ridley, and 3 green) were recaptured by the contract vessel. Additionally, 31 recaptures and recoveries (26 loggerhead, 4 Kemp's ridley, and 1 green turtle) were reported by other investigators.

Loggerhead turtle, *Caretta caretta*—Seven hundred and seventy-four loggerhead turtle captures were recorded off the east coast of Florida. Loggerhead turtles captured in Florida ranged from 38.2 to 110.0 cm SSCL (Fig. 2). Eighty percent (n=616) of the loggerhead turtles captured were subadults and 20% (n=153) were adults. Loggerhead turtles were present year-round in the Cape Canaveral area (Table 2). Total monthly captures were highest during November, December, and January; however, yearly captures for these months varied substantially. Subadult loggerhead turtles were most abundant during all months, except June, and showed a decrease from April to July as adult abundance increases, probably in response to the nesting season (Fig. 3). Peaks in the relative composition of the adult size class during April and June correspond to the peak densities reported by Henwood (1987a) for males and females, respectively.

Sixty-eight tagged loggerhead turtles have been recaptured or recovered since the implementation of this study. Fifty-two (76%) of these turtles were initially tagged by the contract vessel. The remaining sixteen (24%) loggerhead turtle recaptures were tagged by other investigators in Florida and Georgia.[1,2,3,4,5,6,7] Methods of capture included shrimp trawl (69%), beach stranding (22%), pound net (3%), power plant intake canal (3%), nesting female (1%), and SCUBA sighting (1%). The amount of time between tagging and recapture ranged from 1 to 2,499 days. However, 70% (n=45) were recaptured within a year of initial capture.

Twenty-six loggerhead turtles (23 subadults and 3 adults) initially captured

Table 1

Monthly trawling effort (net hours) and turtle capture rates (turtles/net hour) for 1989–91. Cc=*Caretta caretta*, Lk=*Lepidochelys kempi*, and Cm=*Chelonia mydas*.

Year and month	Effort (net hours)	Total turtles/hr	Species		
			Cc/hr	Lk/hr	Cm/hr
1989					
Jan	77.0878	0.3113	0.2465	0.0130	0.0519
Feb	13.1918	0.2274	0.0758	0.1516	0.0000
Mar	25.9838	0.3079	0.2694	0.0385	0.0000
Apr	41.1742	0.2672	0.1700	0.0971	0.0000
May	103.9350	0.0770	0.0673	0.0096	0.0000
June	123.1230	0.1462	0.0568	0.0650	0.0244
July	70.7558	0.1555	0.1555	0.0000	0.0000
Aug	72.7545	0.1924	0.1787	0.0137	0.0000
Sept	19.9875	0.1001	0.1001	0.0000	0.0000
Oct	49.5690	0.0202	0.0202	0.0000	0.0000
Nov	139.1130	0.1006	0.0719	0.0216	0.0072
Dec	56.3648	0.1242	0.1064	0.0177	0.0000
1990					
Jan	27.1830	0.1104	0.1104	0.0000	0.0000
Feb	57.5640	0.2258	0.1737	0.0521	0.0000
Mar	18.3885	0.4894	0.4350	0.0544	0.0000
Apr	23.1855	0.4313	0.3450	0.0863	0.0000
May	15.9900	0.7505	0.5003	0.2502	0.0000
June	31.5802	0.1583	0.1583	0.0000	0.0000
July	147.1080	0.1971	0.1971	0.0000	0.0000
Aug	117.5265	0.0851	0.0851	0.0000	0.0000
Sept	—	—	—	—	—
Oct	—	—	—	—	—
Nov	148.3072	0.1483	0.1079	0.0067	0.0337
Dec	127.5202	0.2666	0.2196	0.0235	0.0235
1991					
Jan	28.7820	0.8686	0.7991	0.0695	0.0000
Feb	23.1855	0.2588	0.2588	0.0000	0.0000
Mar	35.9775	0.1946	0.1668	0.0278	0.0000
Apr	21.5865	0.2780	0.2780	0.0000	0.0000
May	92.7420	0.2588	0.2588	0.0000	0.0000
June	33.5790	0.4467	0.4467	0.0000	0.0000
July	35.7776	0.6988	0.6988	0.0000	0.0000
Aug	21.9862	1.0916	1.0916	0.0000	0.0000
Sept	—	—	—	—	—
Oct	43.3089	0.2309	0.2078	0.0231	0.0000
Nov	77.5515	0.0903	0.0903	0.0000	0.0000
Dec	106.7332	0.1031	0.1031	0.0000	0.0000
Total	2,028.60				

[1] Bolten, A. University of Florida, Archie Carr Center for Sea Turtle Research, Gainesville, FL, 32611. Personal commun., 1992.

[2] Foster, K. National Marine Fisheries Service, Miami Laboratory, 75 Virginia Beach Drive, Miami, FL, 33149. Personal commun., 1994.

[3] Guseman, J. University of Central Florida, Department of Biology, Orlando, FL, 32816. Personal commun., 1992.

[4] Henwood, T. National Marine Fisheries Service, Southeast Regional Office, 9450 Koger Boulevard, St. Petersburg, FL, 33702. Personal commun., 1991.

[5] Martin, E. Applied Biology, Inc., P.O. Box 974, Jensen Beach, FL, 34958. Personal commun., 1991.

[6] Stuntz, W. National Marine Fisheries Service, Pascagoula Laboratory, P.O. Drawer 1207, Pascagoula, MS, 39567. Personal commun., 1991.

[7] Teas, W. National Marine Fisheries Service, Miami Laboratory, 75 Virginia Beach Drive, Miami, FL, 33149. Personal commun., 1991.

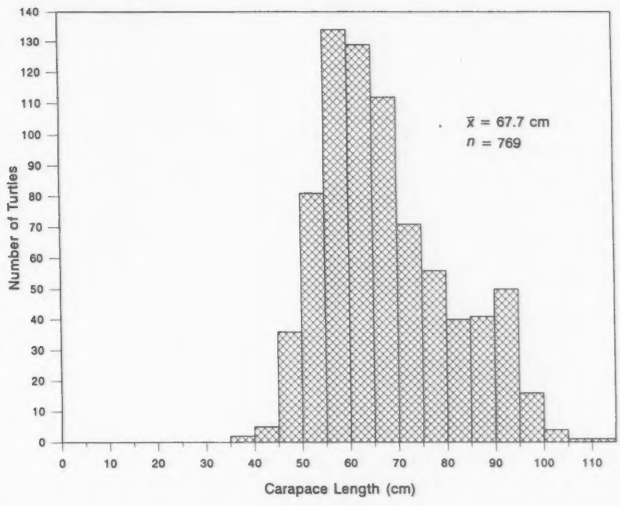

Figure 2

Length frequencies of loggerhead turtles, *Caretta caretta*, collected along the Atlantic coast of Florida from 1986 to 1991.

Table 2

Monthly and yearly trawl captures of loggerhead turtles, *Caretta caretta*, in the nearshore waters of Florida. Dashes indicate trawling effort outside of the study area.

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
1986					1	5/—	—	—	—/5	37	31	44	123
1987	90	47	31	12	5	11/—	—	—	—	—/3	2	0	201
1988	1	0	16	1	2	0	4	3	2	6	8	15	58
1989	19	1	7	7	7	7	11	13	2	1	10	6	91
1990	3	10	8	8	8	5	29	10	10	10	16	28	145
1991	23	6	6	6	24	15	25	24	—	9	7	11	156
Total	136	64	68	34	47	43/—	69/—	50/—	19/—	66/—	74	104	774

within the Cape Canaveral study area were subsequently recaptured within this area. Of this total, eleven turtles (9 subadults and 2 adults) were captured and recaptured in the Port Canaveral ship channel. Eight loggerhead turtles captured in the Cape Canaveral area during the winter were recaptured or recovered in Georgia, North Carolina, and Virginia during the summer and fall (Fig. 4). All these turtles were subadults ranging from 51 to 61 cm carapace length. Three tagged loggerhead turtles (two subadults and a nesting female) were reported south of Cape Canaveral by fishery-independent sources.

There was a stronger correlation between carapace width and carapace length for subadult loggerhead turtles (r=0.9612; n=508) than for adults (r=0.7724; n=151). Regression equations were computed for the relationship of carapace width (CW) to length ($SSCL$) for subadults:

$$CW = 9.0289 + 0.6848\ (SSCL);$$

and adults

$$CW = 22.9153 + 0.5052\ (SSCL).$$

Fifty-one yearly growth rates were calculated for forty-nine loggerhead turtles. Extrapolating annual growth rates from these data is difficult owing to the

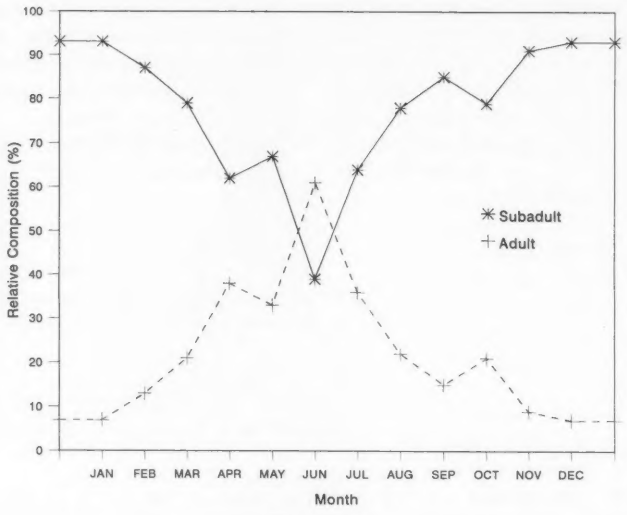

Figure 3

Relative composition of subadult and adult loggerhead turtles collected along the Atlantic coast of Florida from 1987 to 1991.

small sample sizes, measurement errors, and short-term recaptures. A number of treatments were applied to the growth data in an attempt to control for measurement error. However, no single approach was able to account for all the error. Consequently, growth rates were calculated for 1) all data combined, 2) those tag and recapture data recorded by the contracted personnel, and 3) those data with recapture intervals greater than 90 days. A mean growth rate of 5.56 ± 23.91 cm/yr (range: –11.49 to 167.17 cm/yr) was calculated for all loggerhead turtle recaptures. Analysis of the loggerhead turtles tagged and recaptured by the contract personnel indicated a mean growth rate of 1.00 ± 1.23 cm/yr (range: 0.00 to 4.01 cm/yr). Additionally, a mean growth rate of 2.98 ± 7.12 cm/yr (range: –5.96 to 38.44 cm/yr) was calculated for all loggerhead turtle recaptures greater than 90 days at large, 1.84 ± 1.76 cm/yr (range: –0.23 to 8.08 cm/yr) for all recaptures greater than 180 days at large, and 1.77 ± 1.88 cm/yr (range: –0.23 to 8.08 cm/yr) for all recaptures greater than 360 days at large.

The von Bertalanffy growth interval equation was fitted to each of these data treatments. Estimates of asymptotic length (a) for loggerhead turtles ranged from 96.08 cm to 112.52 cm and estimates of intrinsic growth rate (K) ranged from 0.0365 to 0.0588 (Table 3). The growth model for captures and recaptures by the contract vessel had the lowest residual mean square, a criterion commonly used to select the best fit growth model (Dunham, 1978). The estimated parameters for this data treatment (a=112.52 cm; K=0.0365) are similar to Henwood's (1987b) estimated parameters for nonlinear regression of the von Bertalanffy equation (a=110.002 cm; K=0.0313).

Kemp's ridley turtle, *Lepidochelys kempi*.—One hundred and thirteen Kemp's ridley turtle captures were recorded on the Atlantic coast of Florida. Kemp's ridley turtles ranged in size from 21.5 to 60.3 cm SSCL (Fig. 5). Sixty-five percent (n=70) of these turtles were early to mid-subadults (20–40 cm). With the exception of a single adult turtle, the Kemp's ridley turtles caught on the east coast were classified as immature. Kemp's ridley turtles were captured year-round in the Cape Canaveral area (Table 4). Their presence in the Cape Canaveral area appeared to be seasonal; 61% (n=69) of the turtles were captured during the winter months of December to March. However, a relatively large number of Kemp's ridley turtles captured in January and February of 1987 and in March of 1988 contributed significantly to this trend. Captures of Kemp's ridley turtles during the following years did not exhibit a pronounced seasonal pattern.

Nineteen tagged Kemp's ridley turtles were recaptured or recovered. Eighty-nine percent (n=17) of the turtles were recaptured by shrimp trawls, and 11% (n=2) were recovered from beach strandings. With the exception of a single turtle captured in the area

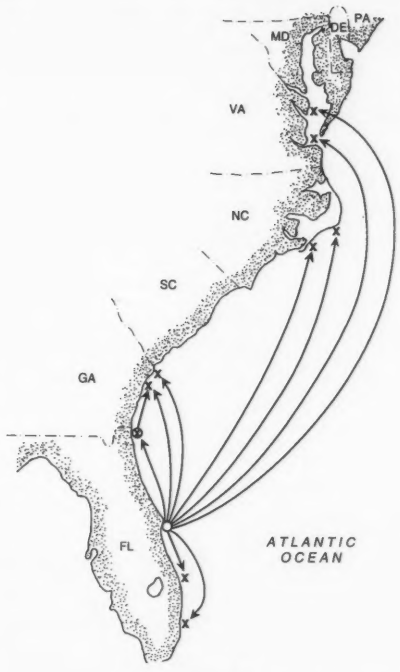

Figure 4

Long-distance recaptures and recoveries of tagged loggerhead turtles. Note: Arrows are not intended to indicate routes traveled by tagged turtles; they are a visual aid to differentiate tagging and recapture sites. O=tagging location; X=recapture location.

of initial tagging after 615 days at large, all Kemp's ridley turtles were recaptured within a year.

Twelve (63%) Kemp's ridley turtles were initially captured in the Cape Canaveral area and subsequently recaptured within this area. Of this total, four turtles were captured and recaptured in the Port Canaveral ship channel. Two Kemp's ridley turtles had multiple recaptures within the Cape Canaveral area, one tagged in November 1986 was caught once in December 1986 and again in May 1987. Another turtle tagged in May 1990 was recaptured twice that September.

Six Kemp's ridley turtles exhibited long-distance movements to and from the Cape Canaveral area (Fig. 6). Two of the recaptures were NMFS Galveston Laboratory headstart turtles released offshore of Padre Island, Texas, in May and captured on the Atlantic coast of Florida in February and March, 0.73 and 1.88 years after release.[8] Two Kemp's ridley turtles displayed seasonal movements northward. The turtles were originally tagged in the Cape Canaveral area in December and February and subsequently recovered in Georgia and South Carolina in July. Another Kemp's ridley turtle exhibited a southerly migration along the Atlantic coast, from Virginia Beach to Port Canaveral.[9]

There was a strong correlation between carapace width and carapace length (r=0.9953; n=105) for Kemp's ridley turtles. Regression of carapace width on length resulted in the equation

$$CW = -2.7157 + 1.0288\,(SSCL).$$

A straight-line equation was applied to the length-weight data; however, graphical analysis of the residuals indicated a curvilinear relationship between the two variables. Power regression was performed through the log-log transformation of weight and length measurements. A strong correlation (r=0.9756; n=88) was calculated for the transformed weight (WT) to length relationship, regression of these variables resulted in the equation

$$\log WT = -8.2837 + 2.8444\,(\log SSCL).$$

Twelve yearly growth rates were computed for ten Kemp's ridley turtles. A mean growth rate of 8.28 ± 9.81 cm/yr (range: 0.00 to 29.16 cm/yr) was calculated for all Kemp's ridley turtle recaptures. Analysis of the Kemp's ridley turtles tagged and recaptured by the contract personnel indicated a mean growth rate of 6.92 ± 9.36 cm/yr (range: 0.00 to 29.16 cm/yr). A mean growth rate of 8.79 ± 10.32 cm/yr (range: 0.00 to 29.16 cm/yr) was calculated for recaptures greater than 90 days at large and 5.94 ± 1.80 cm/yr (range: 4.26 to 7.84 cm/yr) for recaptures greater than 180 days at large.

The von Bertalanffy growth interval equation was fitted to each of these data treatments. Estimates of asymptotic length ranged from 60.66 cm to 77.85 cm and estimates of intrinsic growth rate ranged from 0.0577 to 0.6037 (Table 5). Asymptotic lengths were probably underestimated because of the lack of adult-sized Kemp's ridley turtles in the database.

Green turtle, *Chelonia mydas* —Forty-one green turtles, ranging in size from 24.0 to 55.4 cm SSCL (Fig. 7), were taken from Florida waters. Eighty-one percent (n=33) of the green turtles captured off the east coast of Florida were early subadults less than 40 cm SSCL. No adult green turtles were encountered.

[8] Caillouet, C., Jr. National Marine Fisheries Service, Galveston Laboratory, 4700 Avenue U, Galveston, TX, 77551. Personal commun., 1991.

[9] Keinath, J. Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA, 23062. Personal commun., 1993.

The presence of subadult green turtles in the Cape Canaveral area appeared highly seasonal (Table 6); 73% (n=30) were captured from November to January. As with other turtle species, this pattern resulted from a high number of captures in 1987 and a high monthly variation during other years.

Four tagged green turtles were recaptured during this study. Three turtles were originally tagged by contract personnel in the Cape Canaveral area. The tag codes for the fourth green turtle matched a set of NMFS tags that had been distributed to Fort Lauderdale, Florida. Of the three green turtles tagged at Cape Canaveral, one was initially tagged in January 1987 and then recaptured in April, approximately 68 km to the north. Another green turtle that was initially tagged in the Cape Canaveral area in January 1990 was recaptured in this area the following October. A third green turtle was captured off Port Canaveral in September 1990 and found stranded approximately 41 km to the north the following month.

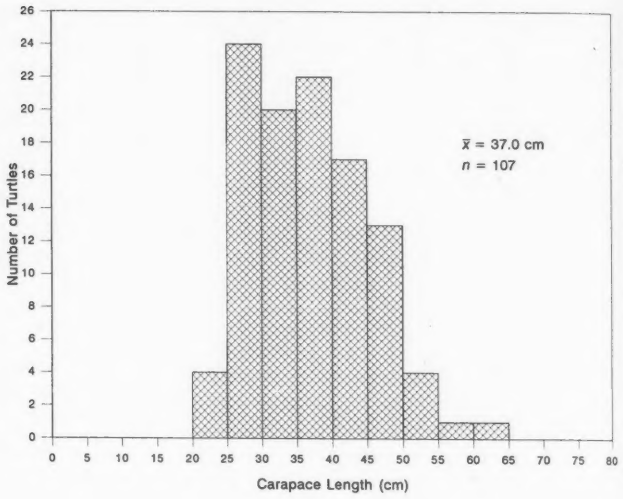

Figure 5

Length frequencies of Kemp's ridley turtles, *Lepidochelys kempi*, collected along the Atlantic coast of Florida from 1986 to 1991.

There was a strong correlation (r=0.9590; n=39) between carapace width and carapace length for green turtles. Regression of carapace width on carapace length resulted in the equation

$$CW = 4.1763 + 0.6847\ (SSCL).$$

Residual analysis of the length-weight relationship indicated the need for curvilinear terms in the regression model. A strong correlation (r=0.9587; n=37) was calculated for the log-transformed weight to length relationship, and regression of these variables resulted in the equation

$$\log WT = 8.8784 + 2.9815\ (\log SSCL).$$

There were no growth data available for green turtles owing to the relatively low number of recaptures and the lack of data for the recoveries.

Table 3

Estimated values of asymptotic length (a) and intrinsic growth rate (k) from nonlinear regression of von Bertalanffy growth interval equation for loggerhead turtles (one asymptotic standard error in parentheses).

Data treatment	n	a	k
All recaptures	51	96.08 cm (7.07)	0.0586 (0.0149)
		Residual mean square error = 9.1054	
Capture/recapture by contract vessel	17	112.52 cm (24.74)	0.0365 (0.0204)
		Residual mean square error = 0.4088	
All recaptures >90 days	33	96.09 cm (8.72)	0.0588 (0.0185)
		Residual mean square error = 13.8969	
All recaptures >180 days	24	96.40 cm (7.97)	0.0569 (0.0162)
		Residual mean square error = 10.8231	
All recaptures >365 days	19	96.10 cm (8.82)	0.0573 (0.0183)
		Residual mean square error = 13.5947	

Discussion

The data for this project were collected incidentally through the commercial shrimp fishery of east-central Florida. Bias in trawling effort oc-

Table 4

Monthly and yearly trawl captures of Kemp's ridley turtles, *Lepidochelys kempi*, in the nearshore waters of Florida. Dashes indicate trawling effort outside of the study area.

	Jan	Feb	Mar	Apr	May	June	July	Aug.	Sept	Oct	Nov	Dec	Total
1986	1[1]				1	0/—	—	—	—/0	1	1	4	8
1987	13	10	4	1	0	1/—	—	—	—	—/1	1	2	33
1988	1	0	17[1]	0	0	0	2	1	1	1	2	2	27
1989	1	2	1	4	1	8	0	1	0	0	3	1	22
1990	0	3	1	2	4	0	0	0	4	1	1	3	19
1991	2	0	1	0	0	0	0	0	—	1	0	0	4
Total	17	16	24	7	6	9/—	2/—	2/—	5/—	5/—	8	12	113

[1] Recapture of a NMFS Galveston Laboratory Headstart Kemp's ridley turtle.

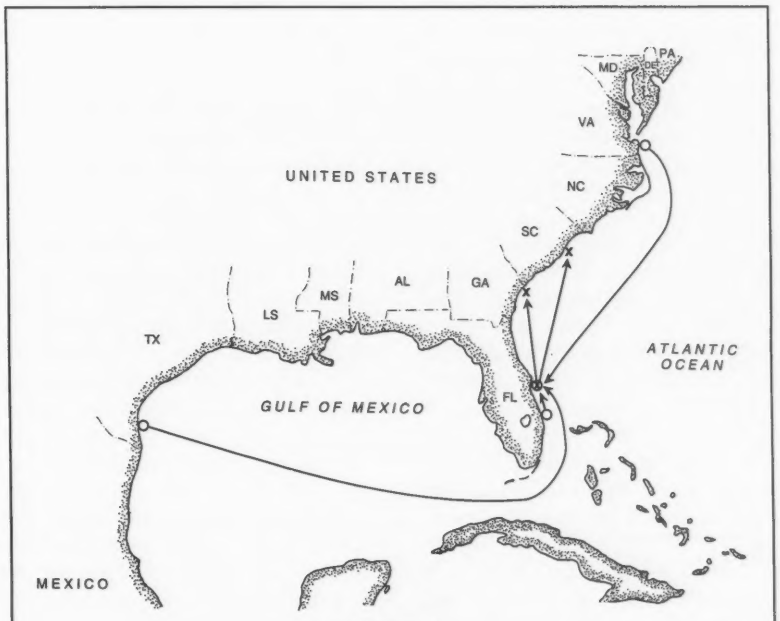

Figure 6

Long-distance recaptures and recoveries of tagged Kemp's ridley turtles Note: Arrows are not intended to indicate routes traveled by tagged turtles; they are a visual aid to differentiate tagging and recapture sites. O=tagging location; X=recapture location.

curred because amount of effort and location of trawling were a function of shrimp abundance. These factors certainly contributed to the annual, and possibly seasonal, variation in turtle captures observed in this study. Restrictions imposed on the shrimping industry during the course of the study also affected data collection. There was a marked reduction in the number of loggerhead captures following NMFS regulations issued in June 1987, requiring the use of TED's in the Cape Canaveral area (Federal Register, 1987). Despite the bias and inconsistencies of data collection, the use of trawl gear allowed access to marine turtle developmental stages not encountered in nesting beach surveys and inshore netting studies. Furthermore, the Cape Canaveral area has a history of trawl studies for general comparison.

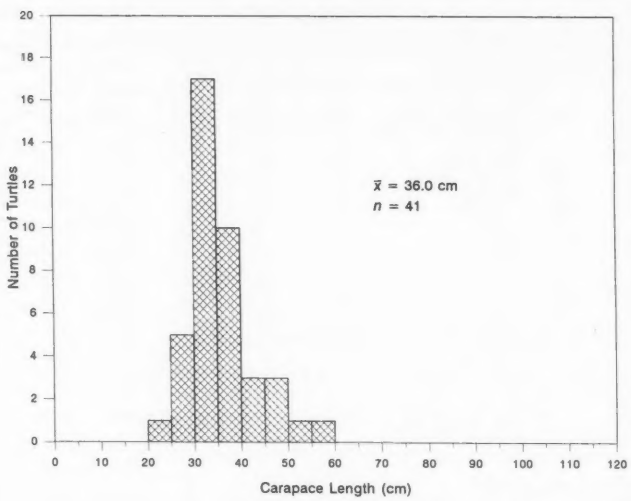

Figure 7

Length frequencies of green turtles, *Chelonia mydas*, collected along the Atlantic coast of Florida from 1986 to 1991.

Trawl surveys conducted by NMFS and the U.S. Army Corps of Engineers from 1974 to 1984 established that marine turtles, especially loggerhead turtles, aggregate in the Port Canaveral ship channel. Henwood (1987a) reported a loggerhead CPUE of 2.00 to 4.86 turtles/hour from July to October 1980, increasing to 12.05 turtles/hour in November. Butler et al. (1987) noted that mean CPUE by month was greater than 10 loggerhead turtles/hour from November 1981 to March 1982 and that there were lower CPUE values from April to September 1982. The CPUE values cited from the previous studies are greater than those in the present analysis, which may be attributable to the different objectives of the present study and the former trawl surveys. The CPUE data presented by Henwood (1987a) were collected during trawl surveys designed to reduce turtle mortality from maintenance dredging in the Port Canaveral ship channel. Butler et al. (1987) conducted surveys of the channel to develop methods of estimating loggerhead abundance. Marine turtles were the target species in both of these studies. The CPUE values reported in this study should be viewed as representative of turtles taken in the commercial shrimp fishery of east-central Florida.

The data on loggerhead turtles collected in the present analysis are similar to the earlier investigations of Henwood (1987a). Most of the log-

Table 5

Estimated values of asymptotic length (a) and intrinsic growth rate (K) from nonlinear regression of von Bertalanffy growth interval equation for Kemp's ridley turtles (one asymptotic standard error in parentheses).

Data treatment	n	a	K
All recaptures	12	61.11 cm (5.43)	0.0577 (0.2176)
	Residual mean square error = 3.4359		
Capture and recapture by contract vessel	10	60.81 cm (5.76)	0.5943 (0.2439)
	Residual mean square error = 4.1701		
All recaptures >90 days	6	60.66 cm (8.04)	0.6037 (0.3549)
	Residual mean square error = 8.2325		
All recaptures >180 days	3	77.85 cm (21.09)	0.2466 (0.1770)
	Residual mean square error = 1.1478		

Table 6

Monthly and yearly trawl captures of green turtles, *Chelonia mydas*, in the nearshore waters of Florida. Dashes indicate trawling effort outside of the study area.

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
1986					1	0/—	—	—	—/0	0	0	2	3
1987	7	1	0	1	0	0/—	—	—	—	—/0	0	6	15
1988	0	0	0	0	0	0	0	0	1	0	0	2	3
1989	4	0	0	0	0	3	0	0	0	0	1	0	8
1990	0	0	0	0	0	0	0	0	1	3	5	3	12
1991	0	0	0	0	0	0	0	0	—	0	0	0	0
Total	11	1	0	1	1	3/—	0/—	0/—	2/—	3/—	6	13	41

gerhead turtles captured on the east-central coast of Florida were mid-subadults (50–70 cm), typical of immature loggerhead turtles in the western Atlantic Ocean. There was a shift to a larger size class during the spring and summer, a period when reproductively active adults immigrate to the nesting beaches of southeast Florida (Henwood, 1987a). At that time, some immature loggerhead turtles emigrated to foraging grounds as far north as Chesapeake Bay. Captures of adult loggerhead turtles in the coastal waters of Florida declined by late summer with the end of the nesting season. Conversely, the presence of subadult loggerhead turtles increased with the onset of winter.

Kemp's ridley turtles in the northwestern Atlantic are transported from their natal beaches in Mexico by major oceanic currents in the Gulf of Mexico (Collard and Ogren, 1990). The smallest Kemp's ridley turtles captured on the east coast of Florida coincide with the minimum size class for postpelagic turtles in the Gulf of Mexico (Ogren, 1989). Skeletochronological age estimates indicate that these turtles may be two years old (Zug and Kalb, 1989), which may indicate the length of this species' pelagic developmental stage. Recapture data from the present analysis and that of Henwood and Ogren (1987) suggest that Kemp's ridley turtles on the Atlantic coast overwinter in the Cape Canaveral area and migrate to northern foraging grounds during the summer. The lack of significant numbers of adult turtles in the northwestern Atlantic suggests that Kemp's ridley turtles migrate from the U.S. east coast upon reaching sexual maturity. Recently, a Kemp's ridley turtle captured and tagged on the southeast coast of Florida was observed nesting in the western Gulf of Mexico.[10]

Relatively low numbers of green turtles have been captured in the Cape Canaveral area. This observation may be the result of this species preference for a habitat other than the Port Canaveral ship channel and adjacent areas of shrimp trawling. Capture data from fishery-independent studies indicate that early subadult (20–40 cm) green turtles inhabit the nearshore reef tracts off the southeast coast of Florida (Ernest et al., 1989; Wershoven and Wershoven, 1989, 1992; Guseman and Ehrhart, 1990). A slightly larger size class of green turtle was captured on the seagrass shoals of the Indian River Lagoon system (Mendonça and Ehrhart, 1982). Seventy-eight percent of the 108 green turtles captured in Mosquito Lagoon were greater than 40-cm carapace length. Furthermore, green turtles captured in the Indian River Lagoon, south of Sebastian Inlet, were significantly larger than those collected on the reefs offshore of Vero Beach (Guseman and Ehrhart, 1990).

There are a number of problems with the growth data presented in this paper. Extrapolating yearly growth rates from short-term recaptures amplifies measurement error. Extremely large and negative growth values were usually the result of short intervals between capture and recapture. Differences in the measuring techniques used by other investigators was a major source of error when computing growth rates. Length measurements are often reported as "straight-line carapace length" when, in fact, there are four possible straight-line carapace lengths: total, standard, notched, and minimum (Pritchard et al., 1983). Standardized methods of measurement, or a definition of the measurement technique and accurate conversions between the various techniques, are necessary for comparisons between studies (Bjorndal and Bolten, 1988). The growth models presented in this analysis should be interpreted cautiously given the forementioned problems with the database.

In conclusion, the results of this study are indicative of the importance of east-central Florida as a developmental habitat for three species of marine

[10] Martin, E. Applied Biology, Inc., P.O. Box 974, Jensen Beach, FL, 34958. Personal commun., 1994.

turtle. The Cape Canaveral aggregation of loggerhead turtles is composed of significant numbers of subadults. Furthermore, the east coast of Florida supports the second largest rookery for this species (Ross, 1982). Subadult Kemp's ridley and green turtles appear to overwinter in the Cape Canaveral area. The eastern seaboard of North America serves as a vital link between the pelagic stage of marine turtle development and recruitment to the coastal-benthic foraging stages. Continued research in coastal waters is essential to the conservation of these threatened and endangered species.

Acknowledgments

This project was initiated and managed in part by Larry Ogren. I am indebted to Captain Eddie Chadwick and the crew of the FV *Mickey Anne* for their diligence in the collection of data. Thanks are also due to Wayne Witzell and Nancy Thompson for constructive comments during manuscript preparation; Wendy Teas and Kellie Foster for stranding and tagging reports; Alan Bolten, Charles Caillouet, Jamie Guseman, Tyrrell Henwood, John Keinath, Erik Martin, and Warren Stuntz for recapture information; Karen Bjorndal for assistance with growth equations and data treatments; and Lisa Gregory for assistance with the Macintosh graphics program SuperPaint.

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Growth rates of captive dolphin, *Coryphaena hippurus*, in Hawaii

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Dolphin, *Coryphaena hippurus*, also known as mahimahi or dolphin fish, are pelagic, predatory fish distributed in tropical and subtropical regions throughout the world (Johnson, 1978; Palko et al., 1982). They are an important resource, supporting commercial and sport fisheries throughout their range (Oxenford and Hunte, 1986) as well as having considerable potential for aquaculture (Hagood et al., 1981; Szyper et al., 1984; Kraul, 1989, 1991).

Gibbs and Collette (1959) and Palko et al. (1982) reviewed the distribution and biology of dolphin, including age and growth data on wild and captive fish. Age and growth of wild (Oxenford and Hunte, 1983) and captive (Uchiyama et al., 1986) fish have been estimated from daily increments on otoliths and scale annuli (Beardsley, 1967; Rose and Hassler, 1968), from modal progression in length-frequency distribution (Wang, 1979), and from fish of known age reared in captivity (Hassler and Hogarth, 1977; Hagood et al., 1981; Szyper et al., 1984; Ostrowski et al., 1989, 1992; Iwai et al., 1992). There is considerable variability in the data, reflecting environmental and nutritional differences associated with experimental designs for captive fish, as well as differences in size, age, and origin of wild fish. In this paper, growth rates of dolphin reared in Hawaii are presented and compared with those of captive and wild dolphin from different populations, as well as other teleost species. The data presented suggest that there are differences in growth rates and morphology between captive and wild dolphin.

Materials and methods

Fish were reared in captivity at The Oceanic Institute, Hawaii, from eggs obtained from wild Hawaiian broodstock fish (F_1; first generation) maintained at Anuenue Fisheries, State of Hawaii. Up to 3 months-of-age juveniles were fed a semi-moist (27.86% moisture) manufactured diet (pellet) twice daily (4% of their body weight per day). The diet contained 53.75% crude protein, 21% crude fat, and a caloric content of 5.13 cal·mg^{-1} (dry matter basis). Between 3 and 9.5 months, fish were fed to satiation once a day on a mixed diet of extruded salmon pellet (Moore-Clarke), frozen squid, and fish. The feed conversion ratio (FCR) is expressed as a ratio between the dry weight of the total amount of food given and the weight gain of live fish.

Up to 3 months-of-age juvenile fish were reared in an outdoor circular tank of 18,800 L (4 m diameter × 1.5 m water column height). After 3 months, fish were transferred to a 28,000-L tank (6 m diameter × 1 m water column height) used for broodstock maintenance. Both tanks had running ambient seawater (25–27°C and 33–35 ppt salinity) at high flow rates (water turnover rate was at 10 tank volumes per day) and under constant aeration.

The initial population was 48 fish, stocked at approximately 3 fish per m^3. Growth data presented correspond to pooled male and female fish periodically sampled during the period studied (1–9.5 months). Data are of individually sampled fish and are not averages. Small fish (1–3 months) were sampled daily. Sampling frequency of intermediate (3–6 months) and large (6–9.5 months) fish was weekly and bimonthly, respectively, owing to difficulties in handling larger dolphin and because there were fewer individuals available for sampling. Since dolphin metamorphosis occurs during the third week after hatching and one month-old fish are fully developed juveniles (Benetti, 1992; Kim et al., 1993), all data corresponding to fish from 1 to 9.5 months-old were combined.

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Results are expressed as:

Absolute growth	$AG = W_2 - W_1$
Absolute growth rate	$AGR = (W_2 - W_1) / (t_2 - t_1)$
Relative growth	$RG = (W_2 - W_1) / W_1$
Relative growth rate	$RGR = (W_2 - W_1) / W_1 (t_2 - t_1)$
Instantaneous growth rate	$IGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$
Specific growth rate	$SGR = (\ln W_2 - \ln W_1) / (t_2 - t_1) \times 100$
Length-weight relationship	$W = a L^b$
VGBM (length)	$L_t = L_\infty (1 - e^{-K(t-t_0)})$
VGBM (weight)	$W_t = W_\infty (1 - e^{-K(t-t_0)})^b$,

where W_1 = initial wet weight of fish; W_2 = final wet weight of fish; t_1 = time at the beginning of an interval; t_2 = time at the end of an interval; L = fork length in cm; a and b are constants; L_t = fork length (cm) at time t; W_t = total weight (kg) at time t; L_∞ and W_∞ = theoretical asymptotic length and weight, respectively; K = constant indicating the rate of change in length and weight (the Brody growth coefficient); t= time; t_0 = time of hatching; and VBGM is the von Bertalanffy growth model (Ricker, 1975, 1979; von Bertalanffy, 1962; Fabens, 1965):

Table 1

Growth rates of captive dolphin, *Coryphaena hippurus*, in Hawaii, fed a mixture of semi-moist manufactured diet[1] (1–3 months) and Moore-Clarke extruded salmon pellets, squid and fish (3–9.5 months).

Parameter	Value
Absolute growth (g)	4,929
Absolute growth rate (g/day)	19.18
Relative growth	70,413
Relative growth rate	273.98
Instantaneous Growth Rate (g)	0.043
Specific Growth Rate (%/day)	4.33
VGBM[2] Asymptotic length (L_∞) (cm)	169.6
VGBM[2] Asymptotic weight (W_∞) (g)	58,417
t_0 (yr)	0.068
K (annual)	0.72
b (constant; Brody coefficient)	3.07

[1] Manufactured diet contained 27.86% moisture, 53.75% crude protein, 21% crude fat and caloric content of 5.13 cal·mg^{-1} (dry–matter basis).

[2] Von Bertalanffy growth model.

Results

Fish grew to 4.93 kg and 75.8 cm from hatching in 9.5 months. Absolute growth rates (AGR's) in weight and length were 19.18 g·d^{-1} and 0.227 cm·d^{-1}, respectively (Table 1). Specific growth rate (SGR) of 1–3 month juveniles was 10% of their body weight per day, decreasing to 4.3% throughout the adult stage.

Most of the data correspond to fish younger than 6 months. The calculation of separate growth curves for males and females was not legitimate because sexual dimorphism could not be detected before that age. Therefore, the growth curves and the von Bertalanffy growth parameters were calculated from combined data for all male and female fish. Growth in length of dolphin was best expressed by a linear relationship (r^2=0.98), though an asymptotic curve also fit the data well (r^2=0.90). From Figure 1 it appears that a linear fit is better because of a weighting of the regression by the smaller fish. Growth in weight was best expressed as a quadratic equation (r^2 =0.98, Fig. 2). The length-weight relationship is expressed by the equation

$$W = 0.00836\, L^{3.07}\ (r^2 = 0.98;\ \text{Fig. 3}).$$

The von Bertalanffy growth models (VBGM) were used to estimate dolphin growth beyond the scope of data measured and, therefore, should be interpreted

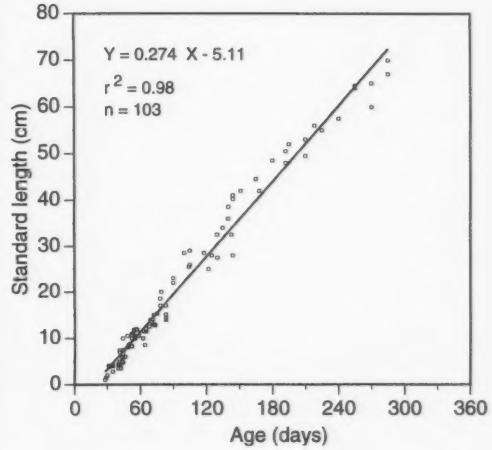

Figure 1

Growth in length of captive dolphin, *Coryphaena hippurus*, in Hawaii.

with caution. The VBGM applied to length-at-age data for captive dolphin in Hawaii is

$$L_t = 169.6 \text{ cm } [1-e^{-0.72\,(t-0.068)}] \text{ (Fig. 4).}$$

The calculated VBGM for weight is

$$W_t = 58.41 \text{ kg } [1-e^{-0.72\,(t-0.068)}]^{3.07}.$$

The feed conversion ratio (FCR) of juveniles up to three months of age was 1.1 (dry feed/live fish), and averaged 1.6 for the entire period studied.

Discussion

There have been several reports of growth rates of wild and captive dolphin, as well as of wild caught fish kept in captivity for various periods of time. Beardsley (1971) reported that a wild caught juvenile kept in captivity grew from one to 35 lb in one year. Schekter (1983) recorded a growth rate of 4.3 kg (from 0.7 to about 5 kg) in 30 days. In Barbados, dolphin may reach lengths of over 80 cm in 5.5 months and over one meter in less than one year (Oxenford and Hunte, 1986). In Hawaii, they also attain a length of over one meter at the end of the first year (Uchiyama et al., 1986). By applying the length-weight regression of Rose and Hassler (1968) to these data, it would correspond to a mass of about 8 kg in one year.

The growth rates presented in this study (4.93 kg and 75.8 cm in 9.5 months) are lower than many of those reported for wild and cultured dolphin in the literature. This may be due to the diet fed to the experimental fish. For instance, Kraul (1989) reported growth rates of 2 kg in 6 months and 9 kg in one year for dolphin that were fed fish and squid in tanks in Hawaii, and of 5.4 kg in 8.7 months for fish reared under identical circumstances but fed commercially available pellets (Kraul and Ako, 1993).

The data suggest that captive dolphin grow slower than their wild counterparts. Yet, even when they are fed artificial diets, growth rates of captive dolphin are among the fastest recorded for teleosts. The specific growth rate (SGR) of dolphin reported in this work (4.3%–10 body weight·d^{-1}), by Ostrowski et al. (1992) (10.7–13.3% bw·d^{-1}), and by Iwai et al. (1992) (9.3–13.0% bw·d^{-1}) are much higher than those of other marine and brackish water fish raised in captivity. For instance, SGR of the common snook, *Centropomus undecimalis*; barramundi, *Lates calcarifer;* hybrid sea bass, *Morone* spp.; Nassau grouper, *Epinephelus striatus;* spotted seatrout, *Cynoscion nebulosus;* red drum, *Sciaenops ocellatus* (Tucker, 1989); three species of mullets, *Liza ramada* (El-Sayed, 1991), *Mugil liza*, and *M. curema* (Benetti and Fagundes Netto, 1991); and the common grouper, *E. guaza* (Fagundes Netto and Benetti, 1984) range from 0.55 to 3.46% of their body weight per day.

The absolute growth rate (AGR) in length of wild and captive dolphin vary between 0.1–0.58 cm·d^{-1}

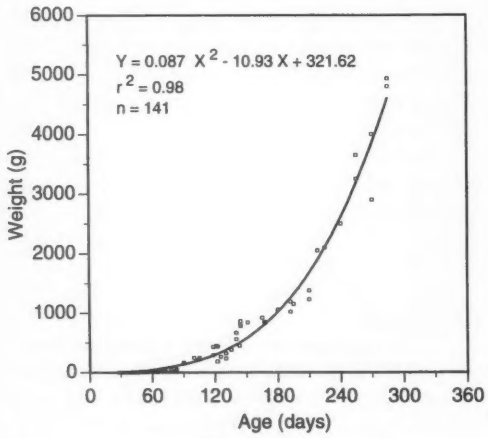

Figure 2

Growth in weight of captive dolphin, *Coryphaena hippurus*, in Hawaii.

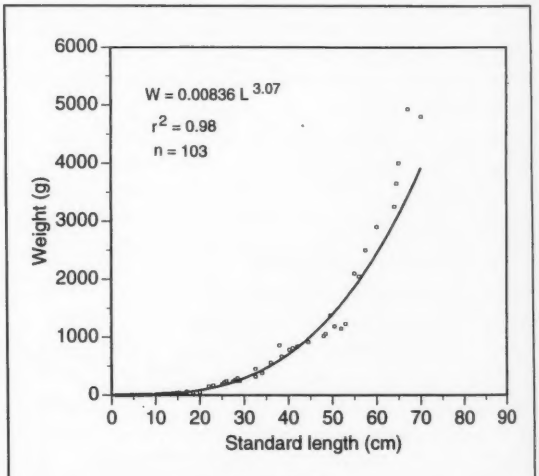

Figure 3

Length-weight relationship of dolphin, *Coryphaena hippurus*, reared in captivity in Hawaii.

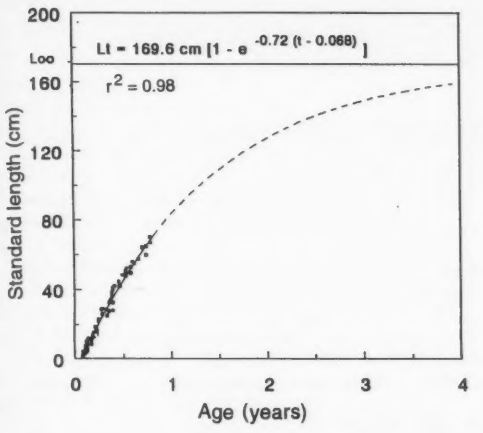

Figure 4

The von Bertalanffy growth model applied to length-at-age data for 1–9.5 month-old captive dolphin, *Coryphaena hippurus*, in Hawaii.

for the first year of life (Oxenford and Hunte, 1983). The AGR value reported in this study (0.227 cm·d^{-1}) is well within this range and the 0.1–0.6 cm·d^{-1} range reported by Brothers et al. (1983) for the Atlantic bluefin tuna, *Thunnus thynnus*, another pelagic teleost. Only a few other pelagic teleosts exhibit growth rates comparable to or higher than dolphin. These include the Atlantic blue marlin, *Makaira nigricans*, and the Atlantic sailfish, *Istiophorus platypterus*, two of the largest North Atlantic pelagic teleosts. Prince et al. (1991) estimated the AGR of young Atlantic blue marlin from otolith microstructure as 1.66 cm·d^{-1}, a value nearly three times higher than that reported for dolphin. From length frequencies of Atlantic sailfish, de Sylva (1957) estimated a maximum absolute growth rate of 1.10 cm·d^{-1}, twice as fast as that reported for dolphin. Although the scope of these comparisons is limited owing to the different age classes of fish, both the blue marlin and Atlantic sailfish exhibit AGR's several times higher than those measured in this work.

In this study, the feed conversion ratio (FCR) was 1.6 (dry feed/live fish). Similarly, Kraul and Ako (1993) obtained a FCR of 1.6 with dolphin fed on a commercially available pellet. FCR's of about 1.0 have been reported for dolphin by Ostrowski et al. (1992), Kraul (1989), and Kraul and Ako (1993), indicating that they are efficient in converting the energy intake from feeds into growth. Relative to other species, dolphin do not appear to require extraordinary food intake to sustain their high growth rates, similar to blue marlin (Prince et al., 1991). In this study, cultured dolphin were fed 4% (dry feed) of their body weight per day. This feeding rate is commonly used for other fish species in captivity, which invariably exhibit slower growth rates and higher FCR. Dolphin appear to exhibit higher energetic efficiency than most other teleosts because they use a proportionally larger portion of the total gross energy ingested for growth and metabolism than for excretion (Benetti, 1992).

Although no spawning was observed, the slight trend of decelerated growth after 180 days (Fig. 1) could be due to the onset of maturation, which in captive dolphin generally occurs in 6 months at 50–55 cm and 2.0–2.5 kg (Kraul, 1991; Ostrowski et al., 1992), but has been observed to occur as early as 3–4.5 months (Uchiyama et al., 1986). Somatic growth rates in teleosts usually decrease after the onset of maturation (Jones, 1976). For dolphin, however, the linear equation fitted the data for the period studied with the highest coefficient of correlation (r^2=0.98). A reason for this may have been the larger sample size during the juvenile stage, when young fish grow very fast. For instance, Hassler and Rainville (1975)[1] found that the length-age relationship of larvae and early juvenile dolphin (13–83 days) was exponential.

The VBGM's were used to model growth beyond the scope of the data and therefore must be considered speculative. Although the data fitted both VBGM's (length and weight) with a high coefficient of determination (r^2>0.98), the asymptotic sizes predicted by the models can not be tested because it has not been possible to keep dolphin alive in captivity for longer than 18 months. In this respect, the age structure of the population should be considered. It is possible that the potential longevity of the Hawaiian dolphin stock may not exceed this maximum age in captivity (about 18 months). For instance, the longevity of dolphin from Florida was estimated to be 4 years, but only 2% of the population was found to be older than 2 years (Beardsley, 1967), and only 4% in North Carolina (Rose and Hassler, 1968). The maximum life span of the Southern Caribbean dolphin stock does not appear to exceed 18 months, and few individuals of the North Caribbean stock live longer than 2 years (Oxenford and Hunte, 1986).

The asymptotic length estimated by the VBGM (L_∞=1.69 m) compares well with existing data for wild fish in the literature (L_∞=1.89 and 1.53 m for males and females, respectively) (Beardsley, 1967). The estimated asymptotic weight (W_∞=58.4 kg), however, is much higher than the maximum weight of 46 kg reported for this species (Florida Sportsman, 1979).

[1] Hassler, N. W., and R. P. Rainville. 1975. Techniques for hatching and rearing dolphin, *Coryphaena hippurus*, through larvae and juvenile stages. Sea Grant Publ. UNC-SG-75-31, 17 p.

This may be explained by the higher value of the exponent (b) of the length-weight relationship calculated for captive dolphin in this study (3.07) compared with wild fish from North Carolina and Florida, $2.58 < b < 2.75$ (Rose and Hassler, 1968). Oxenford and Hunte (1986) reported b values of 2.94 for male and 2.84 for female dolphin from Barbados. These differences indicate that dolphin tend to have shorter, deeper bodies in captivity than in the wild. Blaxter (1988) found that fish reared in captivity tend to be shorter and fatter and have a higher condition factor than fish from the wild, possibly because fish are likely to swim less when confined, diminishing the effect of exercise on growth (Jobling 1990; Christiansen and Jobling, 1990; Benetti, 1992; Christiansen et al., 1992; Boisclair and Tang, 1993). This is consistent with the predictions of the VBGM's in this study, which indicate that it would take longer for dolphin to reach asymptotic length and weight in captivity (6 years) than in their natural environment (4 years or less).

Results presented in this study suggest that captive dolphin grow slower and are less streamlined than in the wild. However, morphological and growth disparities of wild dolphin have been attributed to genetic (Oxenford and Hunte, 1986) and environmental (Rose and Hassler, 1968) differences in unit stocks. The larval development of dolphin from the Gulf of Mexico and from the western Pacific Ocean is similar, but both differ from that off Japan (Ditty et al., 1994). Although Benetti (1992) reported no significant differences between growth rates and development of dolphin larvae in Hawaii from F_1 and F_7 generations inbred in captivity, nothing is known about differences in growth during the juvenile and adult stages among offspring from brood fish from other stocks.

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Modification and comparison of two fluorometric techniques for determining nucleic acid contents of fish larvae

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The ribonucleic acid (RNA) content and the ratio of RNA to deoxyribonucleic acid (DNA) have proven to be reliable indices of the nutritional condition of larval fish (Buckley, 1979, 1980, 1981, 1984; Wright and Martin, 1985; Buckley and Lough, 1987; Clemmesen 1987, 1988; Robinson and Ware, 1988; Canino et al., 1991; Richard et al., 1991; Canino, 1994). Cellular RNA content is correlated with the rate of protein synthesis. DNA content, which remains relatively constant in somatic tissues, may be used as an index of cell number (Bulow, 1987). The RNA/DNA ratio, therefore, reflects the protein synthesizing capability of larval fish and has been used for estimating recent in situ protein growth (see review by Bulow, 1987; Robinson and Ware, 1988; Hovenkamp, 1990; Hovenkamp and Witte, 1991).

Initial methods for determining RNA and DNA concentrations in tissue homogenates, based upon ultraviolet light absorption (Munro and Fleck, 1966; Buckley, 1979), were limited by sample size, requiring about 800 μg dry weight of tissue for a single analysis. The requirement of pooled samples of fish larvae precluded quantitation of variability among individuals. Recent development of highly sensitive fluorometric techniques for direct measurement of nucleic acid contents of marine phytoplankton (Berdalet and Dortch, 1991; Mordy and Carlson, 1991), bacteria (Mordy and Carlson, 1991), and individual fish larvae (Clemmesen, 1988, 1993; Caldarone and Buckley, 1991; Theilacker and Shen, 1993) now provides a greater choice of methods. Several protocols are based upon the fluorescence of the dye ethidium bromide (EB), when bound to nucleic acids. Fluorescence of the nucleic acid-fluorochrome complex is measured before and after digestion of RNA by RNase (Karsten and Wollenberger, 1972, 1977; Robinson and Ware, 1988; Clemmesen, 1993), or after sequential additions of RNase and DNase (Bentle et al., 1981). Total fluorescence is then partitioned into DNA and RNA components and nucleic acid concentrations are calculated indirectly by difference after enzymatic degradation.

A two-dye fluorometric method for nucleic acid analysis of individual fish larvae (Clemmesen, 1988) utilized EB to measure total sample fluorescence and the DNA-specific dye, Hoechst dye H33258 (Hoechst), to measure DNA content directly. A recent modification of this method to a single-dye procedure yields higher DNA estimates and RNA estimates comparable to the previous two-dye method (Clemmesen, 1993). Both protocols require extraction and purification of crude homogenates before analysis. While substances that interfere with sample fluorescence may be reduced or eliminated, the washing and extraction steps of both methods restrict the number of samples that can be processed in a day.

Caldarone and Buckley (1991) developed a two-dye method for determining nucleic acid contents that was coupled with an automated flow-injection analysis (FIA) system in which EB is used to estimate total nucleic acid content and Hoechst is used to measure DNA. This method has the advantage of combining high sensitivity and sample throughput rate with a simple extraction procedure. Unfortunately, an FIA system may be too costly for many laboratories. In this paper, we present an adaptation of the FIA method for conventional static fluorometric analysis (CFA) and compare results using the two procedures.

Methods

Fish larvae and juveniles from laboratory culture and field samples from six species—Atlantic cod, *Gadus morhua*; inland silverside, *Menidia beryllina*; haddock, *Melanogrammus aeglefinus*; tautog, *Tautoga onitis*; winter flounder, *Pleuronectes americanus*; and walleye pollock, *Theragra chalcogramma*—were chosen to provide different species, ages, and nutritional histories for comparison (Table 1). Fish homogenates, except those of walleye pollock, were prepared by homogenizing between 1 and 12 previously frozen individuals with deion-

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Table 1

Fish species, sample abbreviations, and sources used for methods comparison. Fish sampled at discrete ages provided one homogenate at each age. (S) = starved.

Species		Sample name	Source	Age (d)	*n*
Atlantic cod	*Gadus morhua*	C	lab	41–61	3
		C(S)	lab (S)	41–61	3
Inland silverside	*Menidia beryllina*	S	lab	104–108	3
		S(S)	lab (S)	104–108	3
Haddock	*Melanogrammus aeglefinus*	H	lab	11,22,44	3
Tautog	*Tautoga onitis*	T	lab	20	3
Winter flounder	*Pleuronectes americanus*	F	lab	26,41,57	3
Walleye pollock	*Theragra chalcogramma*	P	field	<60	3

ized, distilled water in an Ultra-Turrax homogenizer (Tekmar Co.) with three 15-second pulses at maximum power (Caldarone and Buckley, 1991). Previously frozen walleye pollock larvae were homogenized in prechilled glass homogenizers. Six aliquots of homogenate were pipetted into 1.5-mL polyethylene vials and immediately frozen at –80°C until analysis of three aliquots by FIA and three aliquots by CFA.

Sample extraction procedures followed those outlined by Caldarone and Buckley (1991). Homogenates were extracted in 1% sarcosine (N-lauroylsarcosine), TRIS-EDTA (5.0 mM TRIS-HCl, 0.5 mM EDTA, pH 7.5) buffer for 30 minutes at room temperature, mixed vigorously in a sample vortexer for 10–15 seconds, then extracted for another 30 minutes. Aliquots were diluted with TRIS-EDTA buffer to yield a final concentration of 0.1% sarcosine, then centrifuged at room temperature for 5 minutes at 2,500 times gravity (× g) for CFA and 12,000 × g for FIA. The supernatant was recovered for estimation of nucleic acid concentrations by FIA or CFA. Sarcosine, like other anionic detergents, fluoresces at excitation and emission wavelengths used in the analyses. To reduce this effect, samples extracted in 1% sarcosine required a 10-fold dilution with TRIS-EDTA buffer before FIA (Caldarone and Buckley, 1991). In the modified CFA procedure, a final sample concentration of 0.0125% sarcosine produced an acceptable background fluorescence (blank) value of approximately 5% of the highest nucleic acid standard.

Differences in the total sample volumes required by FIA and CFA procedures required modifications to the concentrations of nucleic acid standards, fluorochrome reagents, and sample volumes of fish homogenates. Nucleic acid standard ranges and homogenate sample volumes were chosen to encompass the typical range of concentrations encountered by each method during routine analysis of individual fish larvae. Working standards of RNA and DNA were prepared as described by Caldarone and Buckley (1991) by serial dilution of previously frozen stock solutions with 0.1% sarcosine in TRIS-EDTA buffer. RNA standards (Sigma Chemical Co., St. Louis, MO, Type IV, calf liver) ranged from 1.97 to 17.68 $\mu g \cdot mL^{-1}$ for FIA and from 0.41 to 13.14 $\mu g \cdot mL^{-1}$ for CFA. DNA standards (Boehringer-Mannheim Corp., Indianapolis, IN, high molecular weight, calf thymus) ranged from 0.16 to 1.52 $\mu g \cdot mL^{-1}$ for FIA and from 0.07 to 2.37 $\mu g \cdot mL^{-1}$ for CFA. Estimates of contamination of the calf liver RNA standard by DNA, determined by fluoresecence in Hoechst, was less than 1% by weight. A 100-μL "spike" of homogenate from walleye pollock larvae was added to serial dilutions of RNA and DNA standards in order to estimate the recovery efficiencies using the CFA protocol.

Fluorochrome working reagents of EB (137.5 $ng \cdot mL^{-1}$) and Hoechst (25 $ng \cdot mL^{-1}$) prepared for FIA in TRIS-EDTA buffer according to Caldarone and Buckley (1991) were modified by reducing the sodium chloride (NaCl) concentration in the Hoechst reagent from 0.2 N to 0.1 N and the pH from 7.5 to 7.0. Working dye solutions of 2.0 $\mu g \cdot mL^{-1}$ EB and 5.0 $\mu g \cdot mL^{-1}$ Hoechst prepared for CFA at the same pH and NaCl concentration as for FIA provided a sensitive linear response to the standards while maintaining a low background fluorescence.

The RNA and DNA concentrations were estimated for each aliquot. In addition, the amount of endogenous fluorescence (sample fluorescence in the absence of fluorochrome dye) was determined for most homogenate samples (21 for FIA, 17 for CFA) by substituting an equal volume of TRIS-EDTA buffer for the fluorochrome working reagent in the assay procedure.

The FIA system for nucleic acid determination is fully described by Caldarone and Buckley (1991). A 50-μL sample is injected into a reagent stream con-

taining one of the fluorochrome dyes. The injected sample is mixed and transported with the reagent to the fluorescence detector which continuously records the fluorescence at 525 nm excitation and 600 nm emission for EB, or 356 nm excitation and 458 emission for Hoechst. The sample fluorescence is displayed as a peak, whose area is proportional to the concentration (Caldarone and Buckley, 1991). Modification of this procedure for CFA required a minimal total volume (sample plus fluorochrome reagent) of 2 mL in order to be measured accurately by the fluorometer (Shimadzu RF–540 spectrofluorophotometer, Shimadzu Corp., Kyoto, Japan), which was adapted to use 12×75 mm borosilicate glass test tubes as cuvettes. For all samples, except the homogenates of larval pollock, a 0.1-mL aliquot of extracted sample was combined with 0.9 mL of TRIS-EDTA buffer and 1.0 mL of fluorochrome working reagent (EB or Hoechst). For larval pollock homogenates, a 0.03-mL aliquot was combined with 0.97 mL of TRIS-EDTA buffer prior to addition of 1.0 mL of the fluorochrome reagents. The sample-fluorochrome mixture was incubated at room temperature for 15–30 minutes before fluorescence was measured at the same excitation and emission wavelengths as in the FIA procedure. Initial trials indicated that maximum sample fluorescence was obtained within 15 minutes and was stable for more than 4 hours at room temperature.

Calculations of nucleic acid concentrations were identical for both methods. First, endogenous sample fluorescence was subtracted from total sample EB or Hoechst dye fluorescence. Sample DNA concentrations were estimated directly from fluorescence in Hoechst dye by using a DNA-Hoechst standard curve. The computed DNA concentration was used to estimate the fluorescence contribution by DNA to the total sample EB-fluorescence by using a DNA-EB standard curve. Fluorescence due to DNA-EB was subtracted from the total sample fluorescence and the remaining fluorescence was assumed to be due to RNA. The RNA concentration was then estimated by using an RNA-EB standard curve.

The relationships between mean RNA and DNA concentration and RNA/DNA ratios of the fish homogenates predicted by FIA and CFA methods were analyzed by using a geometric mean regression procedure (Ricker, 1984) that describes the linear central trend between two independent estimates of the variate.

Results

Standard calibration curves indicated that detection limits for CFA are about 0.07 $\mu g \cdot mL^{-1}$ for RNA and 0.03 $\mu g \cdot mL^{-1}$ for DNA, similar to the values detectable with automated FIA (Caldarone and Buckley, 1991). The precision of both methods was comparable; mean coefficients of variation, V_x (standard deviation as a percentage of the mean), for triplicate determinations from each homogenate averaged 5 to 7% for RNA and 3 to 4% for DNA over a broad range of estimated sample concentrations. Recovery of DNA standards from six replicate "spikes" of larval pollock homogenate with the CFA method averaged 99.5 ± 0.9% in Hoechst and 99.2 ± 2.9% in EB, and recovery of "spiked" RNA standards averaged 94.8 ± 6.0% in EB.

Mean nucleic acid concentrations and RNA/DNA ratios of fish homogenates were generally lower when estimated by CFA relative to FIA (Fig. 1). RNA concentration was most strongly correlated between the two methods and DNA concentration less so (Table 2). The ratio of RNA to DNA was only moderately correlated between the two methods and provided the poorest basis for comparison. Intermethodological calibration between FIA and CFA results was achieved by the application of regression coefficients (Table 2) to mean nucleic acid concentrations and RNA/DNA ratios estimated by CFA (Fig. 2).

Homogenates prepared from larval stages of the gadid species (*Gadus morhua*, *Melanogrammus aeglefinus*, and *Theragra chalcogramma*), tautog, *Tautoga onitis*, and winter flounder, *Pleuronectes americanus*, exhibited negligible endogenous fluorescence, regardless of fluorochrome, over a 3- to 4-fold range of nucleic acid concentrations (Table 3). Endogenous fluorescence was highest for juvenile inland silverside and juvenile winter flounder.

Discussion

Modification of the FIA method described by Caldarone and Buckley (1991) to conventional fluorometry produced an assay protocol with comparable

Table 2

Geometric mean functional regression coefficients describing mean RNA and DNA concentrations ($\mu g \cdot mL^{-1}$ homogenate) and RNA/DNA ratios of 24 fish homogenates determined by conventional fluourometric analysis (CFA) regressed upon estimates obtained by flow injection analysis (FIA).

Variate	Y-intercept	Slope	r^2
RNA	–5.318	0.652	0.972
DNA	–1.795	0.991	0.808
RNA/DNA	–0.309	0.733	0.569

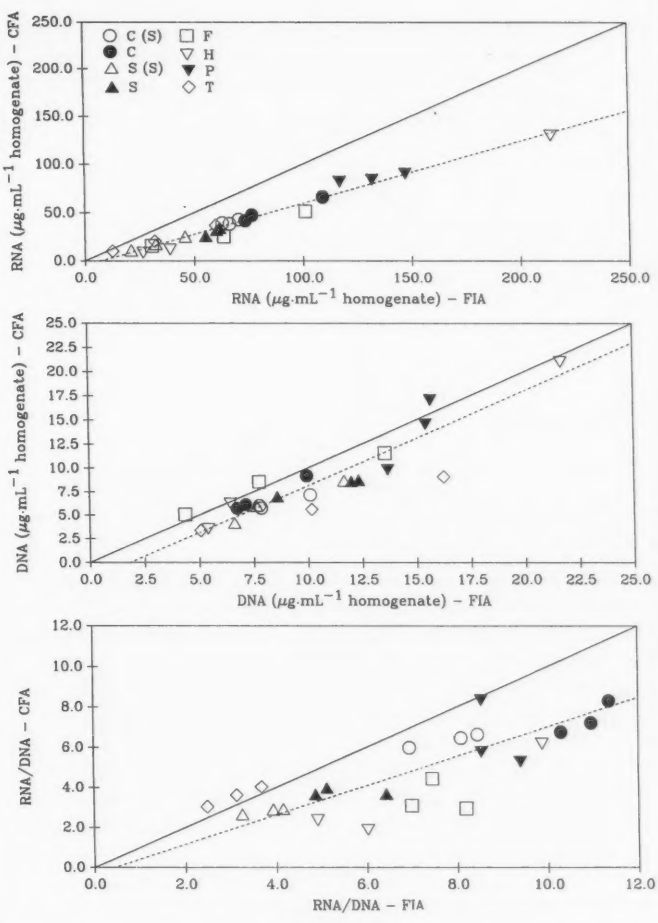

Figure 1

Mean RNA and DNA concentrations and RNA/DNA ratios of 24 crude fish homogenates obtained by flow injection analysis (FIA) versus conventional fluorometric analysis (CFA). Solid line represents a 1:1 correspondence between the two methods. Dashed line is the geometric mean regression between the two estimates. Species abbreviations as in Table 1.

sensitivity, precision, and sample throughput. Mean coefficients of variation (V_x) for DNA concentration estimated by FIA and CFA in this study (3 to 4%) are similar to those reported for replicate assays of a pooled fish homogenate using FIA (Caldarone and Buckley, 1991), another two-dye procedure (Clemmesen, 1988), and a single-dye method (Clemmesen, 1993). In this study, the mean V_x values for RNA concentration were 3 to 5% higher when determined by FIA and CFA for multiple homogenates than for FIA estimates of a single, pooled homogenate (Caldarone and Buckley, 1991) but are comparable to those reported by Clemmesen (1993) for RNA estimates of pooled fish homogenate using a single-dye technique.

The RNA and DNA concentrations of fish homogenates were consistently lower when estimated by CFA compared with FIA. Calibration between the two methods by functional regression relationships established a reasonable basis for comparison of results (Fig. 2), although considerable differences in mean estimated nucleic acid concentrations and RNA/DNA were still evident. We emphasize that sample homo-

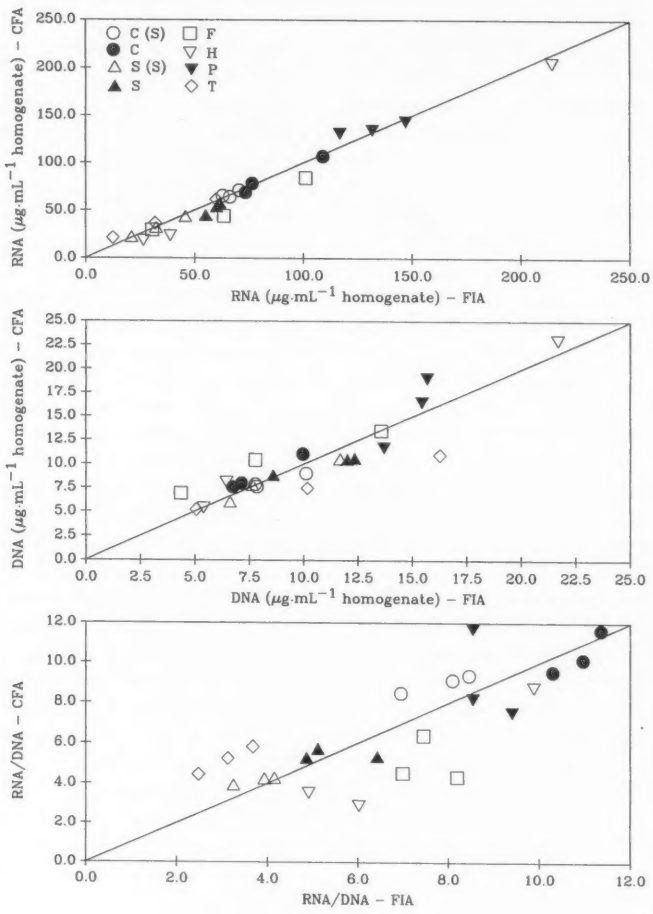

Figure 2

Mean RNA and DNA concentrations and RNA/DNA ratios of 24 crude fish homogenates obtained by flow injection analysis (FIA) versus values transformed from conventional fluorometric analysis (CFA) by functional regression coefficients in Table 2. Solid line represents a 1:1 correspondence between the two methods. Species abbreviations as in Table 1.

genates for this study were chosen from six fish species and assayed across far greater ranges of age and nucleic acid concentrations than reported previously (Clemmesen 1988, 1993; Caldarone and Buckley, 1991; McGurk and Kusser, 1992) in order to broaden the scope of comparison and statistical inference. A comparative study of FIA and CFA techniques within the more limited range of sample concentrations encountered during routine processing of individual larvae of a single species would have undoubtedly yielded higher correlations between estimates.

The FIA and CFA procedures differ primarily in the choice of instrumentation as both use sample extraction by sarcosine. Caldarone and Buckley (1991) reported that nucleic acids in larval fish samples (<200 μg dry weight) were completely extracted in one hour at room temperature. Sample nucleic acid concentrations of fish homogenates in this study were prepared to be similar to those obtained from assays of individual larvae, suggesting that incomplete or differential extraction of homogenates by sarcosine between FIA and CIA is un-

Table 3

Mean endogenous fluorescence[1] of fish homogenates as a percentage of total fluorescence in Hoechst 33258 (Hoechst) or ethidium bromide (EB) fluorochromes. Samples are from larvae unless otherwise noted. (S) = starved.

Species	Sample name	Age (d)	Hoechst		EB	
			FIA	CFA	FIA	CFA
Atlantic cod	C	41–61	<3	<2	<1	3
Atlantic cod (starved)	C(S)	41–61	<3	<2	<1	3
Inland silverside (juvenile)	S	104–108	38	33	<1	12
Inland silverside (juvenile, starved)	S(S)	104–108	40	37	<1	12
Haddock	H	11	<3	<2	<1	<2
		22	<3	<2	<1	<2
		44	<3	<2	<1	2
Tautog	T	20	<3	<2	<1	<2
Winter flounder	F	26	<3	<2	<1	<2
		41	<3	<2	<1	<2
Winter flounder (juvenile)		57	19	14	<1	6
Walleye pollock	P	<60	<3	<2	<1	2

[1] Endogenous sample fluorescence occurs in the absence of fluorochrome dye.

likely. The consistently lower estimates of nucleic acids with the CFA method, relative to the FIA method, implies a lower ratio of fluorescence yield of samples to standards. One possibility is that the effective sample concentration at the detector may be greater in CFA compared with FIA. In FIA, the time between the mixing of dye and sample is precisely controlled but the ratio of dye to sample is unknown. The concentrations of the fluorochrome working reagents used in FIA were increased (14× and 100× for EB and Hoechst, respectively) in order to saturate the standards for the CFA modification. A higher effective sample concentration at the detector (possibly making the samples less available to the dye), coupled with a longer pathlength, may explain the lower fluorescence yield of the CFA samples relative to the standards. The higher correlation between the methods for RNA estimates than for DNA was an unexpected result; RNA content is calculated indirectly (total sample fluorescence minus estimated fluorescence due to DNA) and, presumably, is more subject to measurement error than is direct fluorometric determination of DNA.

Fluorescence by compounds other than nucleic acids represents a potential source of interference in any fluorometric assay. The level of endogenous sample fluorescence should be determined by preliminary analyses when a new fish species or developmental stage is being investigated. Crude tissue homogenates exhibit fluorescence characteristics not found in commercial preparations of nucleic acids that appear to be related to tissue type and developmental stage of the fish. Caldarone and Buckley (1991) found that winter flounder and American sand lance, *Ammodytes americanus*, larvae and the nucleic acids used as standards exhibited negligible amounts of endogenous or residual fluorescence, whereas winter flounder postlarvae and juvenile muscle and liver had levels ranging from approximately 5 to 55% of total fluorescence in EB or Hoechst dyes. Clemmesen (1993) reported endogenous fluorescence values ranging up to 40% in Hoechst determinations of the DNA content of herring, *Clupea harengus*, larvae. In this study, only homogenates of the juvenile inland silverside and juvenile winter flounder exhibited high levels of endogenous fluorescence in Hoechst dye or EB (Table 3), providing further evidence of an ontogenetic effect. Gadid larvae, of approximately the same age as the juvenile winter flounder, displayed negligible amounts of endogenous fluorescence, suggesting that systematic differences may also exist.

Interference in nucleic acid estimation from contaminants of crude homogenate preparations has been reported previously (Brunk et al., 1979; Mordy and Carlson, 1991; Clemmesen, 1993). McGurk and Kusser (1992) compared three fluorescence methods for quantitating nucleic acids in Pacific herring, *Clupea pallasi*, larvae. Of the three, the method incorporating the most extensive purification steps (Clemmesen, 1988) resulted in higher estimates of RNA content and RNA/DNA ratios than the other two methods, suggesting that higher yields may have resulted from the elimination of substances interfering with accurate fluorometric quantitation. However, quenching of nucleic acid fluorescence by contaminants in the samples does not appear to be sig-

nificant in either the CFA or FIA techniques using the sarcosine extraction procedure with larval fish. Recoveries of crude homogenate "spikes" added to nucleic acid standards by FIA (Caldarone and Buckley, 1991) and CFA (this study) are similar to those reported for more purified extracts (Clemmesen, 1993). McGurk and Kusser (1992) reported higher RNA contents and RNA/DNA ratios for yolk-sac herring larvae analyzed with the Clemmesen method (1988) and suggested that fluoresecence absorbance by yolk components may be reduced by the purification steps in that assay. However, when a homogenate of winter flounder yolk-sac larvae was "spiked" with nucleic acid standards and subjected to FIA with a sarcosine extraction procedure, recoveries of calf thymus DNA standards remained unchanged and those for calf liver RNA standards only declined by 3 to 5% (Caldarone, Unpubl. data).

RNA/DNA indices have proven useful as indicators of condition in a wide variety of fish species. When coupled with data on water temperature, and calibrated with laboratory-reared larvae, estimates of recent growth in the field can be obtained (Buckley, 1984). However, this study and others illustrate a common problem with the application of fluorescent techniques to estimation of nucleic acid levels in fish and other biosamples, and the need for intercalibration. Given the disparity in estimates of RNA and DNA contents due to the method of analysis (McGurk and Kusser, 1992; this study) and choice of nucleic acid standards (Caldarone and Buckley, 1991), no direct intermethodological comparisons of data can be made without intercalibration between analytical methods, as done by McGurk and Kusser (1992), Clemmesen (1993), Mathers et al. (1994), and this study. It is inappropriate to compare RNA/DNA ratio values with published data unless the same methods and standards are used. Also, the generalized growth equation in Buckley (1984) uses RNA/DNA ratios determined with an ultraviolet light absorption method that cannot be directly applied to ratios determined with other analytical procedures without running an intercalibration between the two methods. Alternatively, the relation between RNA/DNA, temperature, and growth must be determined for laboratory-reared larvae by using the analytical method of choice before a growth equation can be applied to fish larvae collected in the field.

A general assay protocol for FIA and CFA is presented in Figure 3. For this study, fish larvae were pooled and homogenized to provide adequate replicates for methods comparison. On a routine basis, individual larvae may be frozen in 1.5-mL microcentrifuge vials, then extracted in the same vial, reducing processing time and errors associated with sample transfer. The actual volumes of 1% sarcosine and TRIS-EDTA buffer may have to be determined empirically depending upon the larval fish size and sample volume required for spectrofluorometric analysis. A trained operator can process approximately 80 samples for RNA and DNA determinations as well as standards in eight hours using CFA and in five hours with FIA. We do not routinely assay replicate sample aliquots or correct for endogenous sample fluorescence when preliminary estimates are less than 3% of total sample fluorescence.

The "best" method for nucleic acid analysis of larval fish may well be determined by sample size, instrumentation, the presence of interfering substances, or the need to compare values to previously published data. Flow-injection analysis (Caldarone and Buckley, 1991) is a sensitive, precise assay with a simple extraction procedure and high sample throughput. The modified CFA protocol presented here retains those advantages, extends them to a more inexpensive method using static fluorometry, and provides an intercalibration between the two methods.

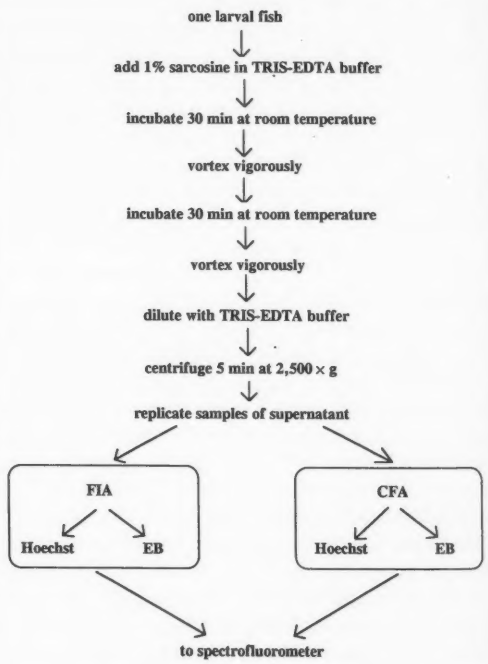

Figure 3

Generalized flowchart of the sarcosine extraction, flow injection analysis (FIA), and conventional fluorometric analysis (CFA) procedures.

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The potential use of otolith characters in identifying larval rockfish (*Sebastes* spp.)

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Rockfish of the genus *Sebastes* are commercially important in the northeast Pacific Ocean, where more than 60 valid species are recognized (Eschmeyer et al., 1983). Although morphological and chromatic characters are used routinely to identify adults of the genus, such traits are frequently ineffective for young of the year, especially larvae. Like most fish, early developmental stages of *Sebastes* spp. have less pigmentation, fewer hard structures (e.g. fin rays), and show less differentiation when compared with adults. However, an ability to discriminate among the larvae of the many rockfish species is critical to the advancement of early life history studies in this group.

A variety of methods have been used to identify larval and juvenile rockfish (Kendall, 1991). Although pigmentation is the most frequently used character (Moser et al., 1977; Laroche and Richardson, 1980; Moser et al., 1985; Kendall and Lenarz, 1987), size at extrusion (Moser et al., 1977), meristic counts (Moser et al., 1977), morphometrics (Morris, 1956), size at specific life history events (Stahl-Johnson, 1985), time of parturition in conjunction with geographic location (Moser et al., 1977), and electrophoretic patterns (Seeb and Kendall, 1991) have all been used to identify larval and juvenile rockfish.

A problem with many of these techniques, however, is that larval characters often undergo ontogenetic change. To overcome this problem, the larvae of many species have been reared in captivity and sequentially sacrificed. This is not only technically demanding, expensive, and time consuming, but differences in development between laboratory and wild fish may affect the number, size, and distribution of the attributes under investigation. Thus, permanent identifiable characters would be useful. A static trait, which retained its characteristics throughout early life, would increase our ability to positively identify rockfish larvae. Otoliths, being acellular aragonitic concretions, are good candidates to retain features produced during the larval stage. Likewise, otoliths have been shown to contain sufficient variation among species (Hecht and Appelbaum, 1982; Akkiran, 1985; Victor, 1987) and stocks (Messieh, 1972; Postuma, 1974; McKern et al., 1974; Neilson et al., 1985; Smith, 1992) to assign with accuracy group membership to individuals.

This study investigates the potential of using otolith microstructure to assist in the identification of larval rockfish. We assume that no change occurs to early larval otolith microstructure once it is deposited (Brothers, 1984; Stevenson and Campana, 1992). Otolith characteristics (nuclear shading patterns, nuclear radius, and first increment width) produced during the early larval period were described and measured from late larval and pelagic juvenile stage specimens of eight species of rockfish: *Sebastes auriculatus*, *S. entomelas*, *S. flavidus*, *S. goodei*, *S. jordani*, *S. mystinus*, *S. paucispinis*, and *S. saxicola*. These species were the most numerous rockfish species collected off the central California coast during the study period.

Methods

Field collections

Samples of young-of-the-year pelagic juveniles and late larvae were collected with a midwater trawl (12 × 12 m) from the National Oceanic and Atmospheric Administration RV *David Starr Jordan*. From 1983 to 1989 nine cruises were conducted off central California (lat. 36°30'–38°10'N) during the months of April-June. Pelagic juvenile rockfish were frozen at sea and returned to the laboratory for final identification. Wyllie Echeverria et al. (1990) have described cruise sampling methodology in detail.

Laboratory procedures

Pelagic juveniles were identified to species from external characteristics, including pigmentation, fin-ray counts, and gill-raker counts (Laidig and Adams, 1991). The sagittal otoliths were removed and affixed whole to microscope slides and were prepared for viewing with the methods outlined in Laidig et al. (1991).

Otoliths were examined with a video image interfaced with a digitizer (Laidig et al., 1991). Distinct reoccurring shading patterns in the

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nucleus (i.e. the otolith core) were noted for each species. Increment counts were made beginning at the first clearly defined mark that completely encircled the primordium (see also Penney and Evans, 1985). This "extrusion" check forms at parturition (Ralston, unpubl. data) and defines the outer edge of the nucleus; the distance from the primordium to this mark is the nuclear radius (Fig. 1), and the increment immediately following the extrusion check is the first growth increment.

Data analysis

The mean radius of the nucleus and the mean width of the first growth increment were compared among species and years. An overall analysis of variance (ANOVA), incorporating separate pairwise *t*-tests, equivalent to Fisher's least-significant difference, was performed to detect differences in the size of the nuclear radius and the width of the first increment among species and years. We used a two-way factorial analysis and calculated least square means (Searle et al., 1980) to evaluate treatment effects among species, years, and the interaction of species and years. A parametric discriminant analysis, assuming a multivariate normal distribution with pooled covariance matrix, was used to determine the percentage of each species that was correctly classified by nuclear radius and first increment width, alone and in combination with each other.

To further evaluate species-specific differences in nuclear radius and first increment width, the data were pooled over years; however, we recognized the difficulty in isolating the effect of species alone. *Sebastes auriculatus* and *S. saxicola* were only sampled in one year; therefore they were not included in the annual variation analysis. For comparison, we treated these single-year studies as the pooled data for the other species.

A blind test was performed to determine the accuracy of the otolith characters in distinguishing between the eight rockfish species used in this study. One hundred otoliths representing all eight species (*S. auriculatus* [*n*=5]; *S. entomelas* [*n*=13]; *S. flavidus* [*n*=15]; *S. goodei* [*n*=14]; *S. jordani* [*n*=25]; *S. mystinus* [*n*=9]; *S. paucispinis* [*n*=11]; and *S. saxicola* [*n*=8]) were given to a reader. No other information (e.g. species, fish length, etc.) about the individual samples was provided. The reader then attempted to identify the correct species of rockfish, using both measured distances and shading patterns. The results of the tentative classification were compared with the actual species identities to determine percent agreement. The significance of this result was evaluated against a null multinomial distribution, by assuming assignments at random to species.

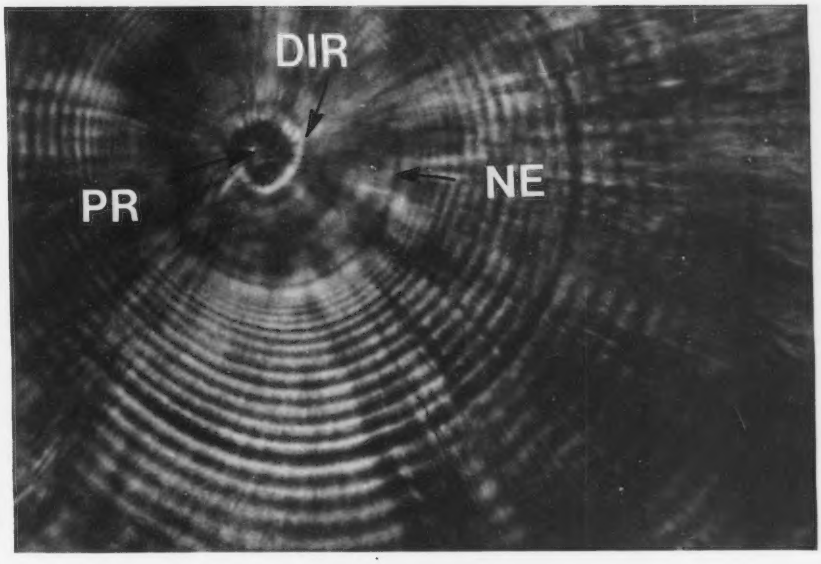

Figure 1

A *Sebastes goodei* otolith displaying the characteristic dark inner ring (DIR) around the primordium (PR). NE = nuclear edge.

Results

Seven specific nuclear shading characters were identified (Table 1): 1) the opacity of the primordium; 2) the opacity of the markings inside the nucleus; 3) the opacity of the increments directly outside the nucleus; 4) the opacity of the nuclear edge; 5) the existence of a dark inner band near the nuclear edge; 6) the existence of a light inner band near the nuclear edge; and 7) the existence of a dark inner ring encircling the primordium.

In some cases, combinations of the shading patterns were sufficient to characterize a species. For example, 84% of the otoliths of *S. goodei* were found to have a dark primordium, a dark nuclear edge, and a dark inner ring surrounding the primordium, while this combination was never found in the other species examined (Fig. 1). Likewise, in *S. jordani*, a light penumbra was regularly found (76% occurrence) adjacent to the inner edge of the nucleus, along with a dark primordium and many inner rings (faint microstructure occurring inside the nucleus). In *S. paucispinis* and *S. flavidus*, there was usually a dark inner band next to the edge of the nucleus (87% and 73% occurrence, respectively) . No annual variation was observed for the nuclear shading patterns. However, in the remaining species, the shading patterns were too variable to establish consistent identifiable character states that would distinguish species.

Annual variations in nuclear radius and the width of the first increment were examined (Fig. 2). Annual variation in nuclear radius among the species was not significant; however, annual variability in the width of the first increment was significant ($P<0.05$). In addition, there was a significant interaction ($P<0.05$) between year and species. The mean width of the first increment of *S. paucispinis*, for example, declined from 1984 to 1989, whereas that of *S. flavidus* increased (Fig. 2A).

Sebastes jordani was found to have the largest average nuclear radius (Table 2; Fig. 2B). The rank order for the remaining species, from largest to smallest average nuclear radii, was *S. goodei*, *S. auriculatus*, *S. paucispinis*, *S. flavidus*, *S. entomelas*, *S. saxicola*, and *S. mystinus*. Individually, the nuclear radii of *S. jordani*, *S. goodei*, *S. auriculatus*, and *S. mystinus* were significantly different ($P<0.05$) from all other species and from each other.

Sebastes jordani was also found to have the largest average first increment of all species studied (Table 2; Fig. 2A). The rank order of the other species, from largest to smallest average widths of the first increment, was *S. paucispinis*, *S. goodei*, *S. auriculatus*, *S. saxicola*, *S. flavidus*, *S. entomelas*, and *S. mystinus*. *Sebastes jordani* and *S. paucispinis* had significantly larger average first increment widths ($P<0.05$) than all other species studied (0.97 μm and 0.91 μm, respectively) (Table 2).

A discriminant analysis was performed on the classification of species by using nuclear radius and the width of the first increment as predictor variables (Table 3). *Sebastes goodei*, *S. jordani*, *S. mystinus*, and *S. paucispinis* were correctly identified from 57 to 83% of the time; *Sebastes auriculatus* and *S. flavidus* were classified correctly 33.9% and 41.8% of the time, respectively. Although these values are less than 50% correct, they represent the largest single classification for each species. Two species, *S. entomelas* and *S. saxicola*, were not often classified correctly (9.5% and 0%, respectively).

In a blind test, the reader correctly classified 70 of the 100 otoliths (70% correct; Table 4), demonstrating that otoliths provide useful information in species identification ($P<0.001$). Greater than 90% of *Sebastes*

Table 1

Observed shading patterns of otolith nuclei for the eight species studied. An F means the attribute was faint, a D means it was dark, and a blank means that it was either variable or did not exist. For the last three attributes, an X means the character was present. aur = *S. auriculatus*, ent = *S. entomelas*, fla = *S. flavidus*, goo = *S. goodei*, jor = *S. jordani*, mys = *S. mystinus*, pau = *S. paucispinis*, and sax = *S. saxicola*.

	Species							
Attribute	aur	ent	fla	goo	jor	mys	pau	sax
Primordium opacity	D	F	F	D	F	D	D	D
Inner ring opacity	F	F		D	F	F	F	
Outer increment opacity	F			D		F		
Nuclear edge opacity	F	F		D		D		
Dark inner band			X				X	
Light inner band			X		X			
Dark primordial ring				X				

goodei and *S. paucispinis* were correctly classified, whereas the remaining species showed lower accuracy varying from 56 to 68%.

Discussion

Otolith characteristics have been shown to vary among species and among stocks. Rybock et al. (1975) and Postuma (1974) used nuclear dimensions to identify different fish stocks. McKern et al. (1974) used otolith dimensions to separate seasonal stocks of steelhead trout, and Victor (1987) used otolith dimensions to separate different species of pomacentrids and labrids. Postuma (1974) related otolith opacity to nuclear size and compared this relationship between stocks. Messieh (1972) used otolith shape to distinguish among stocks of herring. Hecht and Appelbaum (1982) and Gago (1993) also used otolith shape to distinguish between species.

We have found that otolith characteristics can effectively distinguish certain species of *Sebastes*. Four of the species examined, *S. jordani*, *S. goodei*, *S. auriculatus*, and *S. mystinus*, had significantly different nuclear radii from each other and from the other species examined. *Sebastes jordani*, *S. goodei*, *S. paucispinis*, and *S. flavidus* each had unique shading patterns that may help in species identifications. *Sebastes jordani* and *S. paucispinis* were correctly classified over 90% of the time in the blind test, displaying the usefulness of otolith characteristics for identification.

Table 2

Mean nuclear radius and first increment width of otoliths (all years combined) of *Sebastes*. SD = standard deviation. *n* = sample size.

Species	*n*	Nuclear radius (μm)	SD	First increment (μm)	SD
S. auriculatus	56	14.07	1.48	0.82	0.20
S. entomelas	147	11.81	0.94	0.72	0.13
S. flavidus	122	12.09	0.69	0.72	0.16
S. goodei	230	15.15	0.89	0.85	0.17
S. jordani	541	16.96	0.99	0.97	0.23
S. mystinus	106	10.93	1.05	0.65	0.14
S. paucispinis	277	12.20	1.14	0.91	0.18
S. saxicola	12	11.58	0.90	0.73	0.23

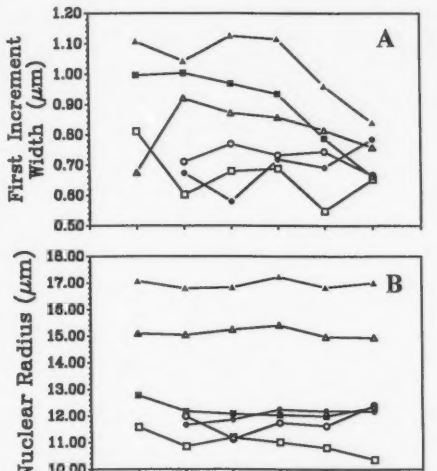

Figure 2

Annual variation in average nuclear radius and first increment width for the six species of *Sebastes* that had multiple year observations. ent = *S. entomelas*, fla = *S. flavidus*, goo = *S. goodei*, jor = *S. jordani*, mys = *S. mystinus*, and pau = *S. paucispinis*.

The specificity of otolith characters gives researchers an opportunity to separate larvae using these characters alone. Without otolith data, the separation of *S. mystinus* larvae from other rockfish larvae is difficult. Although larval *S. jordani* are relatively easy to identify on the basis of pigmentation (Moser et al., 1977), identifications can be confirmed with a few additional otolith measurements.

We suggest from these findings that the difficult task of identifying rockfish larvae can be facilitated in some cases by employing otolith characters in combination with more traditional traits like pigmentation. Of the eight species examined, six species (*S. auriculatus*, *S. flavidus*, *S. goodei*, *S. jordani*, *S. mystinus*, and *S. paucispinis*) had distinctive otolith characters that allowed separation from other species. Many of the larval stages of the more than 60 species of rockfish found in the northeast Pacific Ocean are very similar, and pigmentation alone cannot always reliably separate species. Otolith character examination may be one further method that can aid researchers in accurately identifying species in this group.

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Table 3

Discriminant analysis for nuclear radius and first increment width of otoliths for all years combined showing percent classified as each species of *Sebastes*. aur = *S. auriculatus*, ent = *S. entomelas*, fla = *S. flavidus*, goo = *S. goodei*, jor = *S. jordani*, mys = *S. mystinus*, pau = *S. paucispinis*, and sax = *S. saxicola*.

	Classification							
Species	aur	ent	fla	goo	jor	mys	pau	sax
aur	33.9	0.0	17.9	23.2	10.7	0.0	12.5	1.8
ent	1.4	9.5	38.8	1.4	0.0	22.5	12.9	13.6
fla	5.7	14.8	41.8	0.0	0.0	11.5	18.9	7.4
goo	23.5	0.0	0.9	59.6	14.4	0.0	1.7	0.0
jor	0.9	0.0	0.0	15.9	83.2	0.0	0.0	0.0
mys	0.9	4.7	6.6	0.0	0.9	72.6	6.6	7.6
pau	7.6	2.5	13.7	4.3	0.7	6.5	57.4	7.2
sax	0.0	8.3	25.0	0.0	0.0	33.3	33.3	0.0

Table 4

Results of blind test showing classification rates of *Sebastes* by species category. Bold numbers along the diagonal indicate correct classifications. aur = *S. auriculatus*, ent = *S. entomelas*, fla = S. *flavidus*, goo = *S. goodei*, jor = *S. jordani*, mys = *S. mystinus*, pau = *S. paucispinis*, and sax = *S. saxicola*.

	Classified as:								
Actual	AUR	ENT	FLA	GOO	JOR	MYS	PAU	SAX	% Correct
AUR	**3**	2							60%
ENT	2	**8**			1	2			61%
FLA		1	**9**			1	2	1	60%
GOO	1			**13**					92%
JOR	2			1	**17**	3	1	1	68%
MYS		1	1	1		**5**		1	56%
PAU							**10**	1	91%
SAX		2					1	**5**	63%

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Changes in the spatial patchiness of Pacific mackerel, *Scomber japonicus*, larvae with increasing age and size

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Several investigators have suggested that the spatial patchiness of fish eggs and larvae may be an important factor in the recruitment process (Smith, 1973; Lasker, 1978; Hewitt, 1981; Hcude and Lovdal, 1985; McGurk, 1986). Patchiness has been linked to success in foraging (Hewitt, 1981), ontogeny of schooling behavior (Hewitt, 1981), and predation mortality (McGurk, 1986, 1987). Contagion in the dispersion of ichthyoplankton has been described for Pacific sardine, *Sardinops sagax*, eggs (Smith, 1973); northern anchovy, *Engraulis mordax*, and jack mackerel, *Trachurus symmetricus*, larvae (Hewitt, 1981); haddock, *Melanogrammus aeglefinus*, eggs (Koslow et al., 1985); several taxa found in Biscayne Bay including bay anchovy, *Anchoa mitchilli*, eggs and larvae (Houde and Lovdal, 1985); Atlantic herring, *Clupea harengus harengus*, larvae (Henri et al., 1985); Pacific herring, *Clupea harengus pallasi*, larvae (McGurk, 1987); bluefin tuna, *Thunnus maccoyii*, larvae (Davis et al., 1990); Brazilian sardine, *Sardinella brasiliensis*, larvae; and scaled sardine, *Harengula jaguana*, larvae (Spach, 1990).

The patchy distribution of fish eggs and larvae is initially introduced by the spawning behavior of adult fish. In order to guarantee successful fertilization in a pelagic environment, eggs must be laid when the adults are highly aggregated, and spawning and fertilization must occur almost simultaneously (Hewitt, 1981). Alternately, demersal spawners may deposit their eggs in batches that incubate on a substrate before releasing a cohort of larvae into the pelagic environment (McGurk, 1987). Thereafter, eggs or hatching larvae, or both, disperse, principally in horizontal directions; distribution patterns during this period are primarily influenced by dispersal, diffusion, and transport (Smith, 1973). After a few days or weeks, larvae begin to reaggregate, an activity that becomes more evident in the juvenile stages of most schooling pelagic fishes.

Patchiness-at-age curves for several species of pelagic schooling fishes have been shown to exhibit a characteristic "U" shape: high initial patchiness, followed by a rapid decline as the eggs or newly hatched embryos, or both, passively disperse, followed by an increase in patchiness as the developing fish begin to aggregate in schools (Hewitt, 1981; McGurk, 1987; Spach, 1990). Patchiness-at-age curves for fish eggs and larvae have been interpreted in terms of species-specific differences in life history traits such as adult reproductive behavior and larval feeding ecology, size, growth, and mortality (Smith, 1973; Hewitt, 1981; Koslow et al., 1985; McGurk, 1987). Insight into the function of patchiness may be improved by comparing how patchiness changes with age and size for species with different life histories.

In this note, we present patchiness-at-age and patchiness-at-size curves for Pacific mackerel, *Scomber japonicus*, larvae and compare them with similar curves for other pelagic fish larvae.

Material and methods

Data base

The data used in this work came from the California Cooperative Oceanic Fisheries Investigations (CalCOFI) ichthyoplankton data base. These data are available from the CalCOFI on-line data system (Anon., 1988). Details of station and ichthyoplankton data were published in a series of CalCOFI ichthyoplankton data reports (NOAA Tech. Memo., NMFS, SWFSC, numbers 70–88, 92–100, and 102–105). Size-specific catches of Pacific mackerel larvae, collected from 1953 through 1981, were extracted and summarized for the analyses reported here.

Pacific mackerel larvae were collected with 1-m ring nets from 1953 through 1975 and with bongo nets thereafter. Sampling methods and laboratory procedures were described by Kramer et al. (1972). Out of 23,963 CalCOFI stations sampled from 1953 to 1981, plankton samples from 1,011 stations contained at least one Pacific mackerel larva. The 1,011 stations where larva were collected were assumed to define the Pacific mackerel's

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habitat, and these stations comprised the data set used in the analyses.

Size-frequency analysis

Frequency distributions of larval catches by size were assembled and a negative binomial model was fit to each distribution. The negative binomial has been used to describe aggregated distributions of ichthyoplankton (Hewitt, 1981; Zweifel and Smith, 1981; Smith and Hewitt, 1985). The model is specified by the mean (m) and the index of dispersion (k); the variance (σ^2) is related to m and k as

$$\sigma^2 = m + \frac{m^2}{k}.$$

Lloyd's (1967) index of patchiness (P) was used to describe the intensity of the distribution pattern at various larval sizes where

$$P = 1 + \frac{\sigma^2 - m}{m^2}.$$

The index has been used by several investigators to describe ichthyoplankton patchiness (Smith, 1973; Hewitt, 1981; Houde and Lovdal, 1985; McGurk, 1987) and may be considered as a measure of how many times more crowded an average individual is relative to an individual in a population with the same mean density, but one which is randomly dispersed. The index is independent of density and the scale of sampling (Pielou, 1977; Hewitt, 1982) which allows comparisons of patchiness between relatively abundant yolk-sac larvae and less abundant older larvae. By substituting the expression for the variance of the negative binomial,

$$P = 1 + \frac{1}{k},$$

where k was estimated by using a maximum likelihood estimate expression (Bliss and Fisher, 1953; Smith and Hewitt, 1985). The standard error of the sample estimate of patchiness was estimated by following Lloyd (1967):

$$se(P) \cong \frac{1}{k^2}\sqrt{\widehat{\mathrm{var}(k)}},$$

where $\widehat{\mathrm{var}(k)}$ is the sampling variance of k.

Adjusting for shrinkage and converting to age

Initial size measurements were obtained from larvae preserved in 5% buffered formalin. Preserved size was converted to live size by using the shrinkage rate obtained for jack mackerel larvae from Theilacker (1980). To convert from larval size to larval age, we used the growth curve obtained from laboratory reared larvae with water temperature ranging from 16.8 to 19.2°C (Hunter and Kimbrell, 1980):

$$t = \frac{\ln(SL/3.4432)}{0.05968},$$

where t = age in days since hatching (= 0 age), and SL = standard length in live size (mm). The incubation period (from spawn to hatch) was assumed to be 2.3 days.

Results and discussion

Frequency distributions of larval catches by size are presented in Table 1. The corresponding live sizes, ages, mean abundances per tow, and patchiness parameters are also presented in Table 1. Larvae less than 3.5 mm in length appear to be undersampled in comparison to larger sizes. Pacific mackerel larvae grow rapidly through the first two size classes and therefore are vulnerable to capture for a relatively short period of time; small larvae are also more likely to be extruded through the meshes of the sampling net (Smith and Richardson, 1977).

The change in patchiness with age suggests that recently hatched Pacific mackerel larvae were highly aggregated and dispersed rapidly until approximately five days after spawning (Fig. 1). Patchiness gradually increased with age until 9.2 days, then decreased slightly and continued to increase with age thereafter.

Morphological and behavioral changes of developing Pacific mackerel larvae are summarized in Table 2 and illustrated in Figure 2. A functional visual sensory organ is formed in Pacific mackerel larvae at 6.0–6.5 mm and completed at approximately 8 mm.[1] Although caudal and pectoral fins begin development at 3.5 mm, swimming speed increases rapidly with size only after the pelvic, anal, and dorsal fins are formed at approximately 9.6 mm (Watanabe, 1970; Hunter and Kimbrell, 1980). Hunter and Kimbrell (1980) reported that schooling behavior did not begin until 14 mm, although an increase in patchiness at 4.6 mm is apparent from the plankton catches. It may be that the ontogeny of schooling behavior in Pacific mackerel involves a prolonged period of contact between larvae that is necessary for the successful integration of approach-withdraw and approach-orient behaviors (Shaw, 1960, 1970; Williams and Shaw, 1971). This phenomenon may be statistically recognizable as an increase in patchiness but not visually recognizable as coordinated social behavior.

[1] O'Connell, C. Southwest Fisheries Science Center, Nat. Mar. Fish. Serv., NOAA, P.O. Box 271, La Jolla, California.

Table 1

Size-specific catch statistics for *Scomber japonicus* larvae collected during CalCOFI surveys from 1953 through 1981. A total of 8,396 larvae were caught at 1,011 stations out of a total of 23,963 CalCOFI stations.

	Preserved size (mm)											
	Live size (mm)											
	Age since spawn (days)											
Catch	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.75	6.75	7.75	8.75	9.75
	3.10	3.30	3.50	4.00	4.60	5.20	5.70	6.50	7.70	8.50	9.60	10.70
	2.3	3.0	3.3	5.0	7.2	9.2	10.7	13.0	15.7	17.5	19.5	21.3
Number of larvae/sample												
0	825	671	571	698	774	806	849	836	908	970	980	995
1	93	149	195	143	112	113	90	99	80	26	28	13
2	34	66	81	63	56	35	34	39	9	10	2	2
3–4	28	42	70	52	36	29	23	17	5	3	1	1
5–8	18	31	42	29	18	16	11	15	6	2	0	0
9–16	5	30	27	15	11	8	3	4	2	0	0	0
17–32	4	15	14	10	2	2	1	1	1	0	0	0
33–64	3	4	5	1	1	2	0	0	0	0	0	0
65–128	1	2	4	0	1	0	0	0	0	0	0	0
129–256	0	1	2	0	0	0	0	0	0	0	0	0
Total larvae	709	1,823	2,334	1,088	762	762	350	400	193	67	36	21
Number of samples	1,011	1,011	1,011	1,011	1,011	1,011	1,011	1,011	1,011	1,011	1,011	1,011
Mean per sample	0.701	1.803	2.309	1.076	0.754	0.754	0.346	0.396	0.191	0.066	0.036	0.021
Variance	13.101	67.540	97.629	10.823	13.885	7.421	1.415	1.936	0.933	0.155	0.050	0.036
k	0.092	0.152	0.209	0.189	0.140	0.102	0.139	0.139	0.097	0.048	0.106	0.030
Patchiness	11.91	7.57	5.79	6.30	8.15	10.76	8.21	8.19	11.33	21.98	10.42	34.28
SE (patchiness)	1.09	0.48	0.32	0.44	0.67	0.94	0.91	0.86	1.71	18.42	4.54	39.78

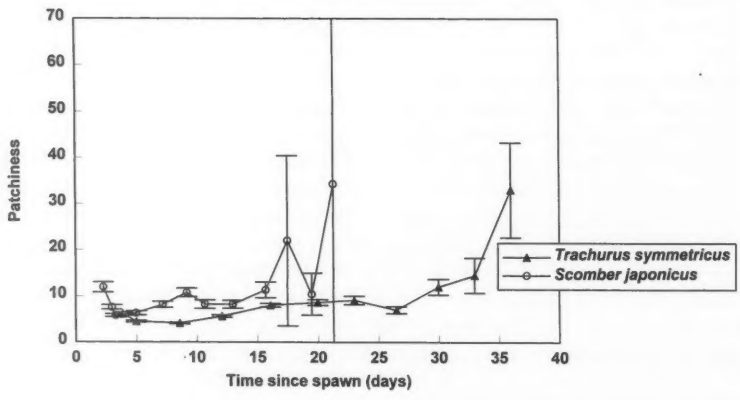

Figure 1

Lloyd's index of patchiness as a function of larval age for *Scomber japonicus* and *Trachurus symmetricus* in the California Current region.

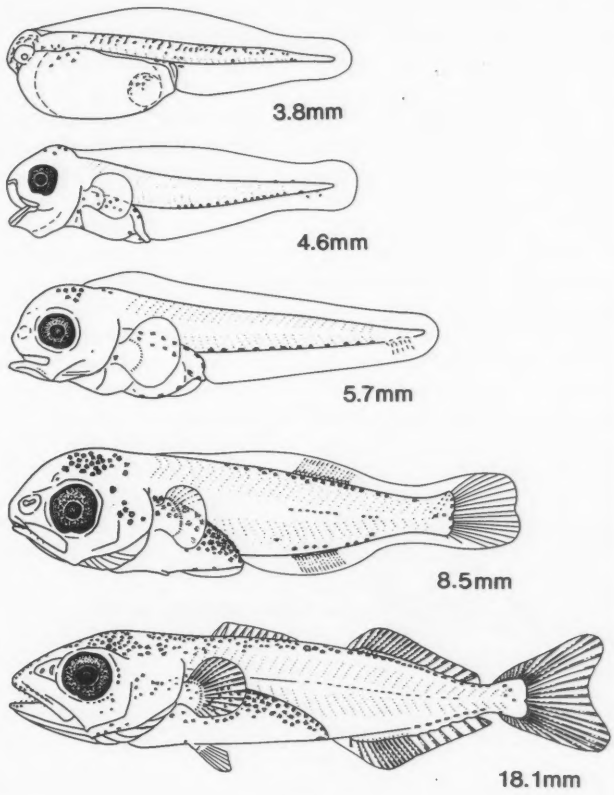

Figure 2

Developmental stages of *Scomber japonicus* larvae (from Matarese et al., 1989). Sizes shown are estimated live sizes.

Table 2

Behavioral and morphological changes in developing *Scomber japonicus* larvae.

	Measurements																
Live size	3.1	3.3	3.5	4.0	4.6	5.2	5.7	6.5	7.7	8.5	9.6	10.7	11.8	12.9	14.0	15.1	16.2	17.3
Swimming speed (cm/sec)[1]		0.4	0.6	0.7	0.9	1.0	1.3	1.8	2.1	2.6	3.1	3.7	4.4	5.0	5.7	6.5	7.3	
Cannibalism[1]									sibling cannibalism between 8 and 15 mm									
Schooling[1]																oriented swimming		
Patchiness		11.9	7.6	5.8	6.3	8.2	10.8	8.2	8.2	11.3	22.0	10.4	34.3					
Feeding ability[2]							Mouth opening and yolk absorption at 4 mm											
Fin formation[2]				CF, PF at 3.5 mm									PvF, AF, DF at 9.6 mm					
Visual sensory organ[3]									developed between 6.5 and 7.7 mm									

[1] Hunter and Kimbrell (1980).
[2] Watanabe (1970); CF = caudal fin, PF = pectoral fin, PvF = pelvic fin, AF = anal fin, DF = dorsal fin.
[3] O'Connell (unpub. data).

Patchiness-at-age curves for six species (*Engraulis mordax* and *Trachurus symmetricus*, Hewitt, 1981; *Clupea harengus pallasi*, McGurk, 1987; *Sardinella brasiliensis* and *Harengula jaguana*, Spach, 1990; and *Scomber japonicus*, reported here) describe a similar sequence: a high index is observed at the youngest larval ages, a low index is observed at one or two weeks after spawn, and thereafter the index increases suggesting the onset of schooling behavior (Fig. 3). The highest index of patchiness at early larval age was observed for *S. brasiliensis* (P=14.5). This can be attributed to intensive spawning behavior of adult sardine, short incubation time (Matsuura, 1983), and fast larval growth (Yoneda, 1987) relative to the other species. The lowest index of patchiness was observed for *C. harengus pallasi* (P=3.5) collected in a small inlet on the west coast of Vancouver Island, British Columbia; McGurk (1987) noted that this may be a reflection-dispersed prey. Houde and Lovdal (1985) reported that fish larvae in Biscayne Bay, Florida, were only slightly more patchy (P=1.3) than their prey, which was abundant and not aggregated (P=1.06). Henri et al. (1985) also reported low patchiness values (P=1.63–3.52) for *Clupea harengus harengus* larvae collected in the St. Lawrence estuary, Quebec. Hewitt (1981) discussed differences in patchiness-at-age curves for *E. mordax* and *T. symmetricus* in terms of their prey availability and foraging strategies. In contrast to *T. symmetricus*, *E. mordax* exhibited an initial high degree of patchiness and slowly dispersed before showing a rapid increase in patchiness at about 18 days of age. *Trachurus symmetricus* larvae were approximately 1/10 as abundant, exhibited lower initial patchiness, and achieved maximum dispersion at an earlier age. *E. mordax* depend on small, but abundant, prey; they have poorly developed swimming capabilities and can effectively forage only through a small volume of water. In contrast, *T. symmetricus* depend on large, but rare, prey items; they have well-developed swimming capabilities and are able to search through relatively large volumes of water.

In comparison to the four clupeoid species, the increase in patchiness was observed to occur at an early age for both *S. japonicus* and *T. symmetricus*. *Scomber japonicus* and *T. symmetricus* larvae share similar morphologies and life history traits. Hunter and Kimbrell (1980) noted that Pacific mackerel larvae may be characterized as having fast growth, rapid swimming abilities, high metabolism, a dependence on increasingly larger prey, and a tendency for cannibalism. Sibling cannibalism may be an important survival strategy for mackerel larvae, where larger individuals prey on smaller ones. Grave (1981) reported that by the time Atlantic mackerel, *Scomber scombrus*, larvae were 12 mm long, 83% of the food items in their diet were other mackerel larvae. High initial dispersal, followed by aggregation of similar-sized larvae may be mechanisms for reducing sibling cannibalism. Although the patchiness-at-age curves for *S. japonicus* and *T. symmetricus* are distinct (Fig. 1), the patchiness-at-size curves are almost coincident (Fig. 4), suggesting that change in patchiness

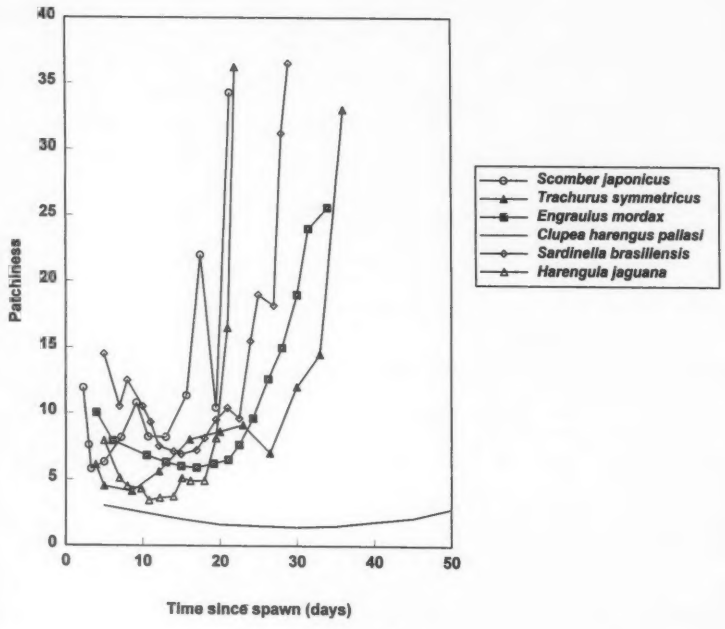

Figure 3

Patchiness-at-age curves for three species from the California Current (*Engraulis mordax*, *Trachurus symmetricus*, and *Scomber japonicus*), two species from southern Brazil (*Sardinella brasiliensis* and *Harengula jaguana*), and one species from British Columbia (*Clupea harengus pallasi*).

may be a size-dependent phenomenom.

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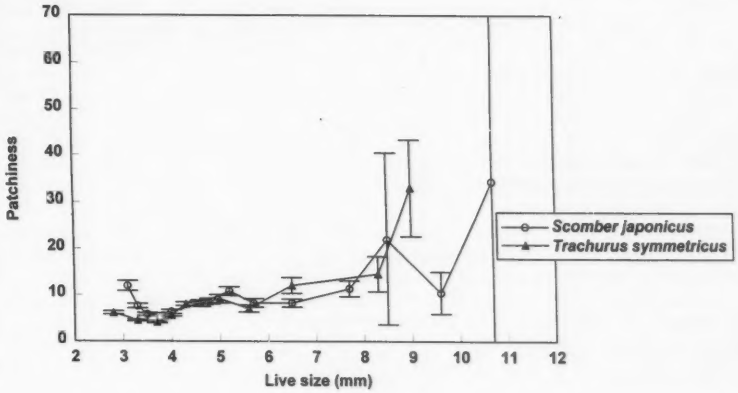

Figure 4

Lloyd's index of patchiness as a function of larval size for *Scomber japonicus* and *Trachurus symmetricus*.

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Growth and morphology of larval and juvenile captive bred yellowtail snapper, *Ocyurus chrysurus**

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The snappers (Lutjanidae) are major components of the reef fish fishery in the Gulf of Mexico (Nakamura, 1976), and recent declines in their populations have prompted interest in a number of management practices including limited catches, size limits, area closures, and additions of artificial reef habitat to improve survival of wild stocks (Leis, 1987; Munro, 1987). Studies on the spawning, distribution, larval and juvenile ecology, and stock assessment of new recruits are crucial to the development of management strategies for reef species since little is known about their early life history (Grimes, 1987). During most developmental stages, snapper larvae are pelagic and widely dispersed, limiting the numbers of specimens to be found in taxonomic collections (Munro, 1987). The similarity in size and pigmentation of small larval lutjanids (<5 mm) and the paucity of species-specific details of size at age and morphological development has made identification of individuals in ichthyoplankton samples difficult (Leis, 1987). Of the fourteen species of snappers that are found in the Gulf of Mexico,[1] larval development has been fully described for only three species: red snapper, *Lutjanus campechanus*, from both laboratory spawned (Rabalais et al., 1980) and wild caught larvae (Collins et al., 1980); gray snapper, *L. griseus*, from wild eggs reared in the laboratory (Richards and Saksena, 1980); and vermilion snapper, *Rhomboplites aurorubens*, from wild preserved specimens (Laroche, 1977). A recent NOAA report by Richards et al.[2] summarizes the larval lutjanid descriptions listed above and introduces some newly available descriptive material for several additional species of snappers including some stages of yellowtail snapper, *Ocyurus chrysurus*. In their report, the yellowtail snapper is included in the genus *Lutjanus*, a change suggested as a result of two recent treatments by Loftus (1992) and Domeier and Clark (1992).

The commercial and recreational importance of snappers has also been recognized by the aquaculture industry, and efforts are underway to culture several of these species in captivity. In this paper we describe the development and growth of laboratory spawned and reared yellowtail snapper. This species is found from Massachusetts through the Caribbean and south to Brazil (Hoese and Moore, 1977). Laboratory culture allowed us to document growth and development of the critical larval and juvenile stages of yellowtail snapper that will aid identification and ageing of larval snappers collected in the field. We have also included information on the effects of a commonly used preservative (ethyl alcohol) on length measurements and pigmentation characteristics of laboratory-cultured larvae for purposes of comparative use with wild-collected larvae.

Materials and methods

Young adult *Ocyurus chrysurus* were collected by hook and line in July 1990 from the Florida Keys and were transported to the laboratory where they were matured and cycled for one year following the methods described by Arnold (1988). Adults began spawning in July 1991 and continued to March 1994.

Eggs were stocked at a density of 50/L in fiberglass tanks (300 and 600 L) with internal biofilters. Larvae were reared at 27–28°C with 12 hours light at salinities of 33–38 ppt on a diet of zooplankton (collected from the Corpus Christi Ship Channel), rotifers (*Branchionus plicatilis*) and brine shrimp nauplii (*Artemia salina*).

The description of larval development is based on larvae from multiple spawns of two different groups of broodstock (15 adults/tank). Larvae were measured live (SL=tip of snout to posterior tip of notochord) to the nearest 0.01 mm on a stereomicroscope equipped with a drawing tube and digitizing

* Contribution 907 of the Marine Science Institute, University of Texas at Austin.

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pad. Drawings were made with a dissecting scope and camera lucida attachment of live, anesthetized larvae before they were preserved in 80% ethyl alcohol (ETOH). To determine laboratory shrinkage rates, the larvae were remeasured after at least one month in ETOH, and preserved lengths were compared to those of the previous, live measurements. Since larvae were drawn from living specimens, no staining or special preparations were required for observation of spines, rays, or other details of morphology.

Results

Pigmentation and overall development

Daily measurements and developmental milestones are presented in Table 1. The pelagic eggs were spherical, averaged 0.96 mm diameter, had a single oil globule, and hatched in 22–24 hours at 27°C. Eggs were essentially transparent, the only pigment observed was a series of small chromatophores on the dorsal surface of the embryo (Fig. 1A). After hatching and through the two day yolk-sac stage, larvae possessed a single unpigmented oil globule in the anterior end of the yolk-sac, an unpigmented finfold, and 24 myomeres. Within 12 hours after hatching (Fig. 1B), the dorsal chromatophores of the embryo had migrated to form a series along the ventral surface of the body and tail; a single stellate chromatophore was present on the gut anterior to the anus, and a light scattering of dark pigment was found on the yolk-sac and lateral surfaces of the head. Exogenous feeding coincided with development of eye pigmentation, functional jaws, and gas bladder inflation at 3.36 mm (age 3 days, Fig. 1C). Pigmentation at this stage included a large dark chromatophore on the ventral surface of the gut, four chromatophores over the dorsal surface of the gut and gas bladder, and a single, dark chromatophore on the ventral tip of the notochord. In live specimens at this stage, we first observed the development of a yellow chromatophore (indicated by arrow on Fig. 1C) located on the lateral surface of the body at about midgut. Larvae 3.67–3.82 mm (Fig. 2A) showed dramatic developmental changes. The development of numerous yellow chromatophores (indicated on illustrations by solid arrows) scattered on the lateral surfaces of the head, gut, and upper body near the base of the pectoral fin, as well as dark stellate chromatophores on the hindbrain and on the ventral edge of the cleithrum coincided with eruption of the pelvic fin buds and the appearance of preopercular spination. Larvae 4.10–4.53 mm (Fig. 2, B–D) were characterized by daily increases in the number of dorsal spines and by elongation of the pelvic fins, as well as by an increase in the density of yellow chromatophores on the lateral head region. Notochord flexion occurred when larvae reached 4.40 mm at 15–16 days posthatch (Fig. 3A) and was followed by full fin formation. Changes in pigmentation consisted primarily of increasingly dense concentrations of yellow pigment on the lateral upper body and head, dark web-like pigment in the membranes of developing fins, and diffuse internal pigment over the gut surface (Fig. 3B). The first indication of adult coloration was visible on early juveniles approximately 14.00 mm SL (Fig. 3C) where yellow chromatophores formed a horizontal line through the eye onto the snout and were also interspersed with the dark chromatophores lining the dorsal and ventral margins of the body at the fin bases. Yellow pigment was also present along the lateral midline of the tail. Near-adult pigmentation was present by 16.00 mm (age 62 days) at which time juveniles were fully scaled (Fig. 3D).

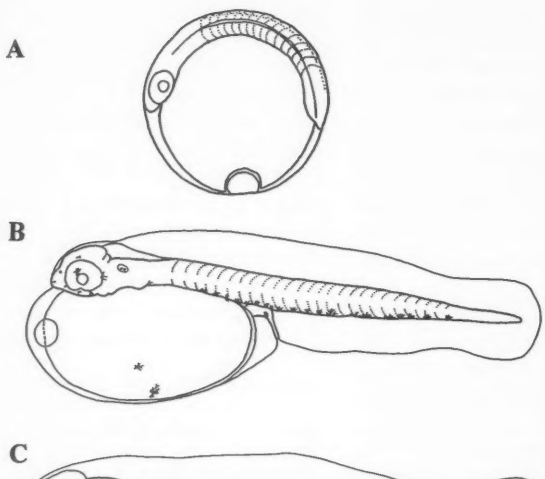

Figure 1

Early developmental stages of yellowtail snapper, *Ocyurus chrysurus*, illustrated from live specimens. (**A**) late embryo egg, 0.96 mm diameter; (**B**) 2.23 mm SL newly hatched larva; (**C**) 3.36 mm, 3 days posthatch. Dark arrows indicate location of yellow chromatophore.

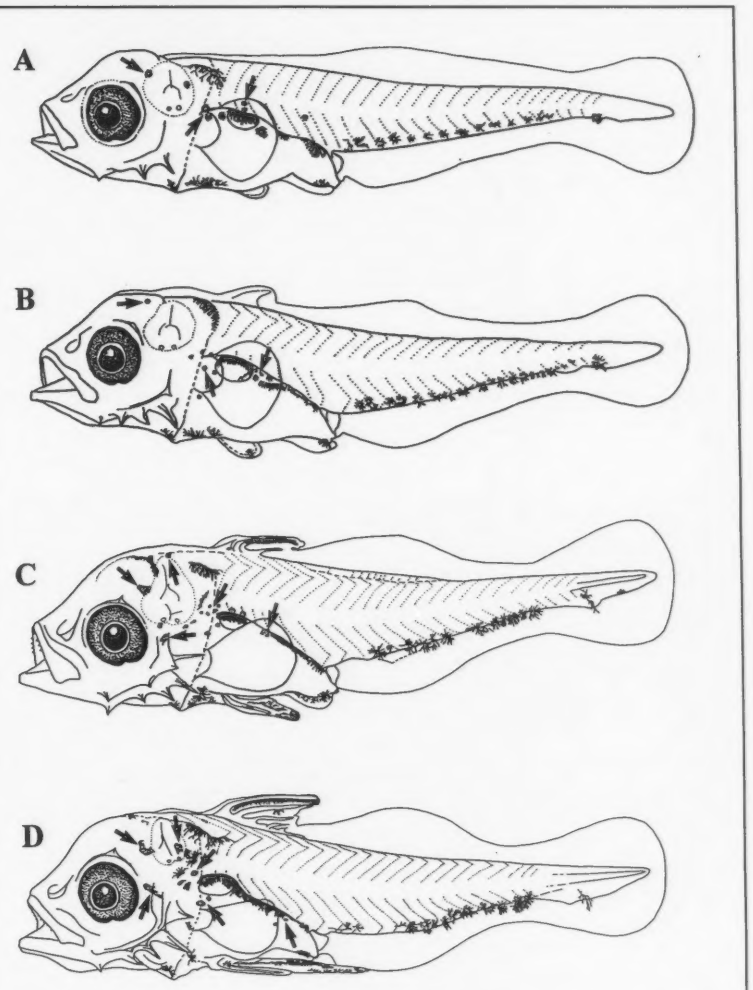

Figure 2

Developmental stages and mean live lengths of yellowtail snapper. Dark arrows indicate location of yellow chromatophores. (**A**) 3.67–3.82 mm SL, days 7-9 posthatch; (**B**) 4.10–4.53 mm SL, days 10-11 posthatch; (**C**) 4.51 mm SL, day 12 posthatch; (**D**) 4.11 mm SL, day 13 posthatch.

Head spination

One or two paired, smooth spines on the posterior edge of the preoperculum first occurred in larvae 3.67–3.82 mm (Fig. 2A). Preopercular spination increased to four and a supraocular ridge with one spine was present at 4.10 mm (Fig. 2B). At 4.50 mm (Fig. 2C), 5 or 6 elongated preopercular spines were present, the longest of which occurred at the preopercal angle. Larvae at 4.80 mm and 16 days of age (Fig. 3A) had a fully formed supraocular ridge with 4 short, smooth spines and 3 supracleithral spines. A reduction in the length of all head spines began at approximately 6.00 mm, but some short, opercular spines remained on the oldest juveniles.

Fin formation

The adult meristic complement of *O. chrysurus* is X+12–14 dorsal, 9 + 8 caudal, I + 5 pelvic, III + 8–9 anal, and 15 or 16 pectoral (Hoese and Moore, 1977). In laboratory-reared larvae, fin development oc-

curred in the following sequence: pelvic and dorsal spines, caudal, dorsal and anal soft rays, pectoral rays (Table 1). The spinous pelvic and dorsal fin formation occurred simultaneously at 4.10 mm. Dorsal and anal fin analage were first visible at 4.51 mm and ray bases were fully formed by 5.35 mm. Caudal flexion and caudal ray formation occurred in larvae between 4.40 and 4.75 mm and was followed by development of the soft rays of the dorsal and anal fins (Fig. 3A); additionally, the spines of the dorsal and pelvic fins were strongly serrated. By 6.23 mm SL, all juveniles had the full adult complement of fin spines and rays (Fig. 3B); however, serrated spines, characteristic of larvae, were still present on juveniles 14.66 mm.

Growth and shrinkage

Laboratory-reared yellowtail snapper showed large variation in size among larvae of the same age (Table 1), and key developmental events were tightly linked to larval size more than to age. Growth rates prior to flexion averaged 0.31 mm/day and decreased to only 0.18 mm/day during the process of transformation to juveniles (4.83–7.00 mm SL, ages 14–28 days). During the last month and a half of recorded development, juvenile growth averaged 0.25 mm/day.

Mean daily lengths of postpreservation larvae are listed on Table 1. Shrinkage after preservation was greatest in larvae prior to any fin development (<4.00 mm), averaging 10.36% through the first 13 days. Larvae with partial fin development (4.83–5.72 mm SL) shrank an average of 9.32%. Once larvae attained complete development of the dorsal and pelvic fins (>5.00 mm); shrinkage was reduced to an average of 7.24% throughout the remaining juvenile stages examined.

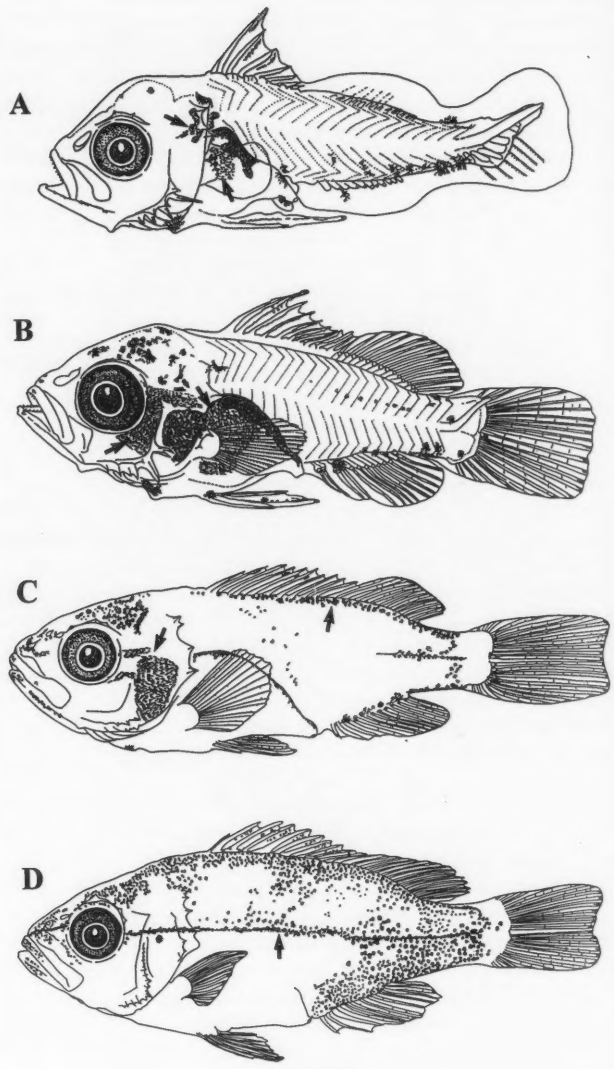

Figure 3

Late developmental stages and mean lengths of yellowtail snapper. Dark arrows indicate location of yellow chromatophores. (**A**) 4.64–4.80 mm SL, days 15–16; (**B**) 6.23–6.73 mm SL, days 18–28; (**C**) 14.66 mm SL, day 31, drawn from preserved specimen; (**D**) 16.05 mm SL, day 62, from preserved specimen.

Discussion

Ocyurus chrysurus have similar larval characteristics to the previously described snappers *Lutjanus campechanus* (Collins et al., 1980), *L. griseus* (Richards and Saksena, 1980), and *Rhomboplites aurorubens* (Laroche, 1977). Preflexion larvae of each of the four species have a series of chromatophores along the ventral midline and have pigment covering the dorsum of the gut and gas bladder. All have

Table 1

Sizes and meristic characteristics of laboratory-spawned and reared larval and juvenile yellowtail snapper, *Ocyurus chrysurus*. All ages listed are in days except for NH = newly hatched < 1 hr old, *n* = number of individuals examined, SD = standard deviation, Shrinkage = % decrease in mean SL after preservation.

	Live SL (mm)					Preserved SL (mm)		Dorsal fin		Pelvic fin		Anal fin		Caudal fin		Pectoral fin	
Age	*n*	Min	Max	Mean	SD	Mean	SD	Spines	Rays	Spines	Rays	Spines	Rays	Principal	Rays	Rays	Notocord flexure
EGG	30	0.92	0.99	0.96	0.02	0.94	0.02	0	0	0	0	0	0	0	0	0	—
NH	42	1.99	2.78	2.23	0.16	2.09	0.23	0	0	0	0	0	0	0	0	0	straight
1	56	2.47	3.60	3.14	0.25	2.85	0.16	0	0	0	0	0	0	0	0	0	straight
2	42	3.09	3.99	3.57	0.26	3.00	0.21	0	0	0	0	0	0	0	0	0	straight
3	39	2.76	3.72	3.36	0.23	3.07	0.20	0	0	0	0	0	0	0	0	0	straight
4	23	3.12	3.68	3.44	0.16	3.05	0.28	0	0	0	0	0	0	0	0	0	straight
5	20	3.24	3.76	3.49	0.18	3.08	0.36	0	0	0	0	0	0	0	0	0	straight
6	23	3.33	3.92	3.64	0.15	3.21	0.28	0	0	0	0	0	0	0	0	0	straight
7	34	3.18	4.22	3.67	0.27	3.35	0.22	0	0	0	0	0	0	0	0	0	straight
8	21	3.51	4.46	3.92	0.25	3.43	0.36	0	0	0	0	0	0	0	0	0	straight
9	13	3.44	4.38	3.82	0.23	3.66	0.46	1	0	1	0	0	0	0	0	0	straight
10	21	3.59	4.76	4.10	0.34	3.67	0.38	1	0	1	0	0	0	0	0	0	straight
11	12	3.88	5.13	4.53	0.37	4.03	0.36	2–3	0	1	0	0	0	0	0	0	straight
12	14	3.50	5.45	4.51	0.59	3.84	0.66	3–4	0	1	0	0	0	0	0	0	straight
13	24	3.77	5.40	4.11	0.35	3.77	0.41	3–4	0	1	0	0	0	0	0	0	straight
15	14	3.95	5.47	4.69	0.57	4.01	0.80	5	0	1	3	0	0	8	4	0	flexed
16	4	3.80	5.52	4.80	0.76	4.59	0.61	5	0	1	3	0	0	9	4	0	flexed
18	6	5.70	6.56	6.22	0.31	5.63	0.33	8	13	1	3	3	9	9	8	0	flexed
21	8	4.80	7.46	5.72	0.86	5.34	0.82	8–9	13	1	4	3	9	9	8	16	flexed
25	11	5.31	8.86	6.69	1.12	6.05	0.82	10	13	1	4	3	9	9	8	16	flexed
28	7	5.68	9.32	7.00	1.28	6.75	1.15	10	13	1	5	3	9	9	8	16	flexed
31	4	7.80	14.66	11.52	3.31	12.19	1.75	10	13	1	5	3	9	9	8	16	flexed
62[1]	5	14.48	17.71	—	—	16.05	1.32	10	13	1	5	3	9	9	8	16	flexed

[1] = Preserved lengths only

large solitary chromatophores on the cleithral symphysis, gut ventrum, anus, and on the notochord at the point of flexure, and all undergo flexion within a narrow size range of 4.2–5.2 mm SL.

There are, however, a few distinctive characteristics that can be used to separate the larvae of these species. Immediately following flexion at 4.40 mm, larvae of *O. chrysurus* and *R. aurorubens* (flexion at 4.7 mm, Laroche, 1977) possess large serrations on both the anterior and posterior margins of the dorsal spines, but these are not present on larvae of *L. campechanus* (Collins et al., 1980) or *L. griseus* (Richards and Saksena, 1980). Preopercular spination is also a useful character in that the longest spine (located at the preopercle angle in each described species) is serrated in *R. aurorubens* but not in *L. campechanus*, *L. griseus* (Laroche, 1977), or *O. chrysurus* (present study). Lyczkowski-Shultz and Comyns[1] compared small, preserved larval *R. aurorubens* and *L. campechanus*, and found that both species had two dorsal spines and 4 or 5 preopercular spines at 3.3 to 3.9 mm SL. Yellowtail snapper differ in having fewer or no dorsal spines and only three preopercular spines at the same preserved sizes (corresponding to days 7–11, Fig. 2, A and B). Lyczkowski-Shultz and Comyns[1] also examined pigmentation differences in <4.0 mm larvae and identified a characteristic pigment spot in *R. aurorubens* located on the branchial chamber and visible through the operculum; they also observed pigment on the anterior surface of the gut (at the level of the pectoral fin base) in *L. campechanus*. Larval *O. chrysurus* of the same size range were devoid of pigment in either of these locations. Larval *R. aurorubens* (Laroche, 1977) had numerous, dark chromatophores located on both the midbrain and hindbrain regions in all sizes of larvae examined, however, the two species of *Lutjanus* and *O. chrysurus* had head pigment only on the hindbrain area. The yellow chromatophores found on live or recently preserved specimens of *O. chrysurus* are definitive characteristics for identification of this species; unfortunately, this light-colored pigment was not visible after the 30-day preservation period in larvae <7.00 mm SL and would not likely be detected in ichthyoplankton samples preserved in ETOH. The yellow chromatophores were faintly visible on the snout, operculum, and lateral line of the larger preserved individuals. Larval *O. chrysurus* can be distinguished from the other described lutjanid species by utilizing combinations of the above characteristics including the presence of heavy serrations on both the anterior and posterior margins of the dorsal spines at the time of flexion, lack of serrations on the longest preopercle spine, reduced number of preopercle spines and dorsal spines at comparable sizes, and lack of internal pigment on the anterior surface of the gut or branchial chamber.

Newly hatched and early developmental stages of larval fishes are rarely collected or retained in net samples.[3] Those larvae that are collected show significant handling effects (Theilacker, 1980; Hay, 1981; McGurk, 1985), including distortion and size reduction that result in less than optimal depictions of size at critical stages of development. In contrast, laboratory-reared specimens provide more realistic size values and information on age and pigmentation not available to studies with field-caught larvae. The shrinkage rates at each age and phase of morphological development in *O. chrysurus* are conservative measures because field-collected larvae show additional shrinkage from net damage. In small unossified larvae, reduction in SL as a result of net collection alone increased shrinkage rates of laboratory-preserved northern anchovy by 19% (Theilacker, 1980) and in Pacific herring by about 8% (Hay, 1981). From these results it is clear that some additional allowance for net shrinkage should be applied to the laboratory-preserved lengths of *O. chrysurus* when compared to those of field-caught individuals; however, shrinkage rates may be variable between species; therefore the value to be used is unclear. Shrinkage rates have been shown to decrease with increasing age and size of larvae (Theilacker, 1980; McGurk, 1985) and to become equivalent to that of larvae exposed to laboratory handling only (e.g. no net damage) once larvae are completely ossified. Shrinkage rates of laboratory *O. chrysurus* also decreased in postflexion larvae, stabilizing at <10% in early juveniles. Therefore, to make predictions regarding the live size or age of field-collected larval snappers, an additional, though unknown, rate of shrinkage due to net damage should be taken into account in preflexion stages but not in postlarvae and juveniles.

The nomenclatural status of the yellowtail snapper has come under review recently. After describing the morphology of the natural hybrid between *O. chrysurus* and *Lutjanus synagris* (Loftus, 1992) and the laboratory-produced hybrids of *O. chrysurus* and *L. synagris* (Domeier and Clarke, 1992), the authors of these studies concluded that the morphological and meristic data indicated that *Ocyurus* is probably not a distinct genus from *Lutjanus*. The larval morphology described in this study of *O. chrysurus* also confirms the very similar size and developmental characteristics of this species with the previously described members of the genus *Lutjanus*.

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Acknowledgments

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Validation of otolith-based ageing and a comparison of otolith and scale-based ageing in mark-recaptured Chesapeake Bay striped bass, *Morone saxatilis*

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Anadromous striped bass, *Morone saxatilis*, populations in the mid-Atlantic region comprise important commercial and recreational fisheries (ASMFC[1]). Stock assessments for these fisheries depend upon age estimates using annular structures of scales (Merriman, 1941; Mansueti, 1961; Kahnle et al.[2]; Hornick et al.[3]). Age estimation for large adults (>91 cm) has been problematic owing to the presence of false annuli and to difficulty in interpreting narrow annuli in peripheral fields of the scale (Scofield, 1928a; Merriman, 1941; Tiller, 1950; Mansueti, 1961). Recent work on otolith microchemistry to decipher environmental histories of migratory striped bass has provided age estimates that were considerably greater than those previously reported (Secor, 1992). Longevity of female Chesapeake striped bass was estimated to exceed 31 years based on examination of otolith microstructure (Secor et al.[4]).

An investigation of the rate of annulus formation in the otoliths of Chesapeake Bay striped bass was performed to verify estimates of growth and longevity (Secor et al.[4]). For otolith microchemistry applications (Secor, 1992), it also was critical to verify that annuli formed at a yearly rate so that seasonal patterns in Sr/Ca ratio (exposure to varying salinity) could be interpreted. Heidinger and Clodfelter (1987) reported yearly rates of annulus formation in otoliths of striped bass from one to four years in age in a midwest reservoir, but no specific measurements of accuracy or precision were presented. No other studies have been published on annulus formation in striped bass otoliths.

A mark-recapture study on hatchery-produced striped bass (Rago et al., 1993; Hornick et al.[3]) provided samples of known-age resident and migratory fish that were 3 to 7 years old. From 1985 to 1992, approximately 5.5 million juvenile striped bass were stocked in Chesapeake Bay tributaries (Rago et al., 1993). All fish were implanted with a binary-coded wire tag which indicated year of origin and provided information on their hatchery source and release date and site. The objective of this study was to verify the rate of annulus formation by comparing annulus counts with the known age of recaptured hatchery fish. A second objective was to compare scale and otolith ages of large striped bass (>91 cm, total length [TL]) to determine the accuracy of age estimates derived from scales.

Methods

Known-age study

Striped bass otolith ageing techniques were verified by using two sets of known-age, coded-wire tagged (CWT) adults. A group of 24 CWT fish was obtained from a collaborative study of migratory striped bass conducted by the Maryland Department of Natural Resources (DNR); the National Marine Fisheries Service; the North Carolina Department of En-

[1] ASMFC (Atlantic States Marine Fisheries Commission). 1990. Source document for the supplement to the striped bass FMP-Amendment No. 4. Atlantic States Marine Fisheries Commission. Prepared by Versar, Inc., Columbia, MD, 414 p.

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vironment, Health, and Natural Resources; and the U.S. Fish and Wildlife Service. Between 6 and 8 February 1993, migratory fish were captured off the North Carolina coast by the crew of the NOAA vessel *Chapman* by means of a 90-ft, 2-seam fish trawl that was towed for a maximum of 30 minutes (Laney and Cole, 1993). A second group of 13 CWT fish was obtained from DNR commercial drift gillnet and poundnet surveys between 5 October 1992 and 23 February 1993. Pound nets and drift nets were located in shoal areas of the upper Chesapeake Bay, off Kent Island, MD.

Fish in the study group had been tagged with binary coded wire tags as age-0+ juveniles. Tags were removed from recaptured fish and decoded by personnel at the U.S. Fish and Wildlife Service laboratory in Annapolis, MD, to determine their ages.

Otoliths were extracted, soaked in 10% sodium hypochlorite solution, rinsed with deionized water, and embedded within a Spurr epoxy (Secor et al., 1991). Transverse sections, approximately 1 mm thick, were then cut through the otolith cores with a Buehler Isomet saw. The sections were mounted on glass slides, polished on 600-grain sandpaper, and polished again on a slurry of 0.3-μm alumina until their surfaces were free of pits and abrasions. Polished sections were viewed under a light microscope, and otolith annuli were counted by two independent readers. Annuli comprised a narrow opaque zone and a wide translucent zone under transmitted light microscopy (magnification at 60 or 150×). Annuli were counted along the sulcal ridge in transverse sections. The otolith ages were compared with each other and with the known age of the fish.

Scale vs. otolith study

Scales and otoliths were sampled from recreational landings of striped bass (>91 cm TL) during the May 1992 Maryland "Trophy Striped Bass Fishery." These fish were assumed to have spawned recently in upper Chesapeake Bay tributaries. Five additional fish, large females (>100 cm) collected in 1991 and 1992 from the Patuxent and Nanticoke Rivers by DNR for hatchery propagation purposes, were included in the comparisons. Scales and otoliths were aged independently by a reader at Chesapeake Biological Laboratory (otoliths) and at DNR (scales). Otoliths were prepared and aged as described above. Scale samples for ageing had been removed from the left side of the fish above the lateral line and below the first dorsal fin. Age was determined from either direct interpretation of the scale's annuli or from acetate impressions of the scales. Otoliths and scales were coded so that fish length was unknown to readers.

Results

Known-age study

Striped bass collected in pound nets and drift gill nets in Upper Chesapeake Bay were 3- to 7-year-old males and females. The migratory hatchery striped bass from offshore samples tended to be older, ranging in age from 4 to 7 years old (Fig. 1).

Agreement between known and estimated age for resident striped bass was 100% for both otolith readers (n=13). Exact agreement between estimated and known age for migratory fish (n=24) was 79% and 87% for reader 1 and reader 2, respectively. All migratory fish were estimated to be within one year of their true age. The mean age difference between readers for migratory fishes was not significantly different from 0 (paired t-test: n=37; P=0.66). The mean absolute difference between ages estimated by reader 1 and known-age, a measure of precision, was estimated at 0.13 years. Precision estimated for reader 2 was 0.08 years. Error in age estimates was not related to fish length or age.

Scale vs. otolith study

Age estimates from scales and otolith sections were not significantly different for fish with otolith-estimated ages of 5 to 11 years (Fig. 2; paired t-test: n=30; P=0.41). However, fish with otolith-estimated ages of 22 to 31 years had scale-estimated ages which were, on average, 9 years less than otolith age estimates (Fig. 2; paired t-test: n=30; P<0.0001).

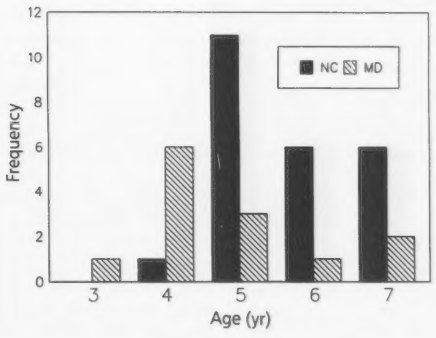

Figure 1

Age-frequency distribution of known-age striped bass from Upper Chesapeake Bay, Maryland (MD) and coastal North Carolina (NC).

Discussion

Annulus formation in otoliths and scales

We verified age estimates of striped bass from annuli observed in sectioned otoliths of fish 3 to 7 years old. Annuli precisely and accurately reflected ages in both resident and migratory Chesapeake Bay striped bass. Annulus appearance at 6 and 7 years was similar to that of annuli from otoliths of much older individuals (Fig. 1 in Secor, 1992). Therefore, we believe that annuli also are formed at a yearly rate in older individuals (e.g. >20 years). Tagged hatchery striped bass represent over 5% of the striped bass population which over-winters in coastal waters off North Carolina (Laney and Cole, 1993). Thus, these fish will continue to provide a pool of known-age material to verify age estimates in older fish. In the next decade it will be possible to verify annulus formation in the oldest fish of the population.

The timing of annulus formation in otoliths was not quantitatively evaluated; samples among months of the year were insufficient to conduct a marginal increment analysis (see Beckman et al., 1988, 1990, 1991). However, we did consistently observe that samples collected during the Maryland Trophy Striped Bass Season in May 1991 and 1992 (see Secor, 1992) contained a newly formed annulus and that no such annulus was observed in otoliths of resident or migratory fish collected in February 1993. Therefore, annulus formation probably occurs during the February–April period. This observation agrees with observations on season of annulus formation in striped bass scales (Merriman, 1941; Heidinger and Clodfelter, 1987).

On the basis of evidence that annuli in otolith ages represent true age in older fish, we believe that scales significantly underestimate age in fish older than 20 years. Scale ages were not significantly different from otolith ages for fish aged 5 to 11 years, ages that corresponded to fish 91 to 110 cm TL. Scale ages were, on average, 9 years less than otolith ages in fish older than 20 years that corresponded to fish >120 cm TL. Because samples were unavailable for ages 12 to 19 years owing to the scarcity of individuals from these year classes (Secor et al.[4]), we could not determine the accuracy of scale ages for this period. In a similar study on southeastern U.S. riverine and reservoir striped bass, Welch et al. (1993) observed that scale ages were in good agreement with otolith ages for fish <90 cm TL but that scale ages were significantly lower than otolith ages for fish 90–10 cm TL. For Sacramento-San Joaquin striped bass, Scofield (1928a) found good agreement between age estimates made from either hardpart for the first eight years of fish life.

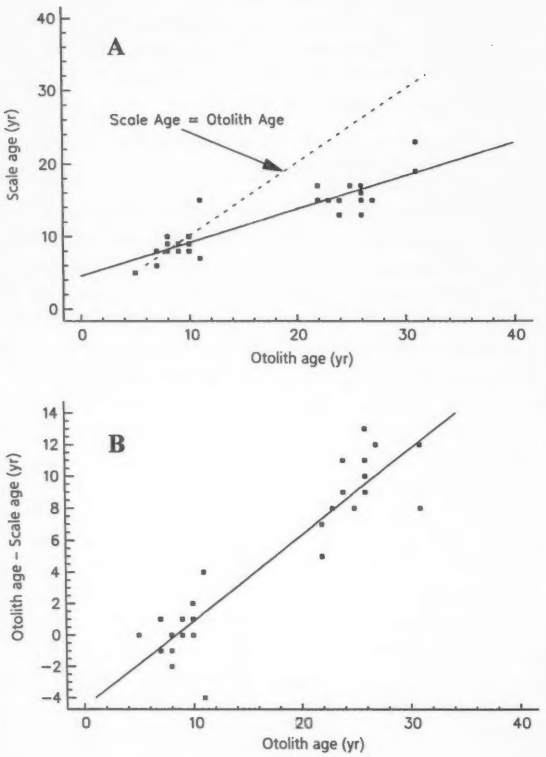

Figure 2

(A) Scale-estimated age vs. otolith-estimated age for Maryland Trophy striped bass (>91 cm TL). Scale age = 4.55 + 0.46 otolith age; n=45; r^2=0.85. For reference, a line corresponding to scale age = otolith age has been plotted. (B) Discrepancy between otolith-estimated and scale-estimated age vs. otolith-estimated age for Maryland Trophy striped bass (>91 cm TL). (Otolith age-Scale age) = –4.55 + 0.54 otolith age; n=45; r^2= 0.88.

Ageing in striped bass stock assessments

Errors in ageing can result in large biases in stock assessments and in mismanagement of fishery resources (Beamish and McFarlane, 1983; Richards et al., 1992). Scientists at the turn of the century recognized that otoliths often provide more accurate and precise estimates of age than do other hard parts (Heinke, 1904; Cunningham, 1905; Haempel, 1910). Indeed, early verification of age estimation based on scales of striped bass relied upon comparisons of age estimates with those based on otoliths

(Scofield, 1928, a and b). However, the popularity and ease of age estimations using scales caused investigators to overlook the importance of verifying ageing methodology (Beamish and McFarlane, 1983).

All stock assessments on migratory populations of striped bass currently rely on interpreting annular features on scales, a largely unvalidated method. Annulus formation in scales of striped bass has been verified for fish up to age three (Humphreys and Kornegy, 1985) and four years (Heidinger and Clodfelter, 1987) in nonmigratory striped bass. Our data indicated that annuli in scales may adequately estimate age for fish less than 12 years of age. Thereafter, scales provided a continuous age distribution between 11 and 20 years of age, and otolith ageing indicated an absence of fish corresponding to these ages (Fig. 2). Therefore, we inferred that otolith-based age determination will provide more accurate estimates after 12 years of age.

A major disadvantage of using otoliths for age determinations is that fish must be sacrificed. Because large and old members of coastal populations have potentially high reproductive values and may be important contributors to annual recruitments (Rago and Goodyear, 1987; Zastrow et al., 1989; Secor et al., 1992; Cowan et al., 1993), it may be undesirable to sacrifice large numbers of these individuals for stock assessment purposes. An alternative approach would be to correct the age estimates from scales by using an otolith vs. scale calibration curve (Fig. 2). Our reported relationship was somewhat variable (r^2=0.85), but with additional otolith samples, reliable prediction of age from scale annuli may be possible.

Acknowledgments

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An evaluation of six marking methods for age-0 red drum, *Sciaenops ocellatus**

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Mark-recapture studies of fishes can reveal valuable information on movement, mortality, and growth rate (Parker et al., 1990). Despite the many successful methods that have been developed for adult fish, marking of small juvenile fishes is problematic (Chapman and Bevan, 1990). Age-0 fish are typically too small and delicate for many marking methods.

A few methods for marking age-0 fishes have been developed: coded wire microtags (Thrower and Smoker, 1984; Brodziak et al., 1992; Bumguardner et al., 1992); spray paint marking (Phinney et al., 1967; Pierson and Bayne, 1983); fluorescent staining (Hettler, 1984; Secor et al., 1991a; Szedlmayer and Able, 1992); and external plastic minitags (Floy Tag and Mfg. Co., Inc., Seattle, WA). All of these methods have advantages and disadvantages based on the attributes of each species. Thus, there is a need to test different marking methods on small size classes of different species to determine the most suitable methods.

Two marking methods have been reported for age-0 red drum, *Sciaenops ocellatus*: 1) spray paint marking,[1] and 2) coded wire microtags (Bumguardner et al., 1992). Fluorescent staining is another method that may be useful for age-0 *S. ocellatus* and has been successfully applied to juvenile red sea bream, *Pagrus major* (Tsukamoto et al., 1989), and to larval and juvenile striped bass, *Morone saxatilis* (Secor et al., 1991a). However, it is not possible from these studies to determine the most useful marking method for age-0 *S. ocellatus*. Hence, we examined mortality, mark retention, and growth in age-0 *S. ocellatus* that were marked by one of six different methods: 1) coded wire microtags, 2) external plastic minitags, 3) alizarin complexone, 4) oxytetracycline dihydrate [OTC], 5) red fluorescent spray paint, and 6) green fluorescent spray paint.

Materials and methods

We marked 614 cultured age-0 *S. ocellatus* (mean standard length [SL] ± standard deviation [SD]=67.4 ± 8.7 mm; range=48–95 mm SL) on 4–5 February 1993. Fish were anesthetized with tricane methanol sulfate (50 mg MS222/L seawater), weighed, measured, and randomly assigned to one of the following treatments: red fluorescent paint (red), green fluorescent paint (green), external plastic tags (plastic), binary coded wire microtags (wire), oxytetracycline-dihydrate (250 mg OTC/L 25 ppt seawater for 15 h), and alizarin-complexone (250 mg alizarin/L 25 ppt seawater for 15 h).

Wire tags were injected into the left epaxial muscle with a specially designed hypodermic needle (Northwest Marine Technology, Shaw Island, WA). The plastic tags (numbered disk: 0.5×3×7 mm) were attached posterior to the dorsal fin with elastic thread sewn through the left and right epaxial muscles. Red and green paints were applied at a pressure of 70 to 100 psi from a distance of 30 to 50 cm (Phinney et al., 1967). A total of 614 fish were marked and classified as follows: 100 OTC, 114 alizarin, 100 red, 100 green, 100 plastic, and 100 wire. All fish were held in a 7,570-L closed seawater system, and differentially marked fish were separated by flow through partitions. Ammonia, nitrite, and nitrate levels were controlled with an oyster shell biological filter (mean ± standard error [SE]: NH_3=0.018 ±0.003 ppm; NO_2=0.253 ± 0.047 ppm; NO_3=47.4 ± 1.9 ppm). Particulates were removed with a sand filter. Salinity was held constant by addition of artificial seasalts or freshwater (mean salinity ± SE=25.0 ± 0.2 ppt). Temperature was held constant with three 1,000-W heaters (mean temperature ± SE=20.8 ± 0.2°C).

Fish mortalities were counted and removed daily for 68 days. All fish were fed daily at 5% body weight per day with Zeigler salmon crumbles no. 2 pellet food (Zeigler Bros. Inc., Gardners, PA). At 25, 48, and 68 days, all fish from each treatment were anesthetized, weighed, measured, and food was adjusted for growth to maintain the daily 5% body weight ration. Red and green paint marks were verified with an ultraviolet light (paint was not visible under white light). Fish were considered marked if at least one granule of pigment was observed. Also, when fish were anesthetized and measured, we randomly sacrificed 20 fish from

* Contribution 8-944903 of the Alabama Agricultural Experiment Station, Auburn University, Mobile, Alabama 36615.

[1] McMichael, Robert H., Jr. Florida Department of Natural Resources, St. Petersburg, FL. Personal commun., 1992.

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Fishery Bulletin 93:191–195 (1995).

each treatment: from OTC and alizarin treatments for otolith mark examination, from wire treatments for wire removal, and from other treatments for future otolith analysis. All samples were preserved by freezing. In estimating percent survival, it was assumed that all fish that were sacrificed (alive and healthy at time of sample) would have survived the 68-d experimental period. The actual survival of sacrificed fish would be lower than 100%, but the difference in survival rate among treatments would be expected to increase if actual survival rates for all fish were known.

Whole sagittal otoliths were removed from OTC and alizarin treatments and viewed with an Olympus BH–2 compound microscope under 100-W ultraviolet light. When fluorescent marks were not visible on whole otoliths, they were sectioned and polished for increased resolution (Secor et al., 1991b; Szedlmayer and Able, 1992). Wire tags were located by making a sagittal incision of the epaxial muscle and examined under a Nikon stereo-microscope. If wire tags were not located by dissection, fish were X-rayed to locate tags.

We compared instantaneous mortality rates with analysis of covariance (ANCOVA) and percent tag retention with a randomized block analysis of variance (ANOVA) with day as blocks and marking method as the factor (Zar, 1984). We compared standard lengths (SL) and weights over marking method with ANOVA for each sample date. If significant differences ($P<0.05$) were detected, we compared the means (ANOVA) or slopes (mortality; ANCOVA) with Newman-Keuls range test (Zar, 1984).

Results

Instantaneous mortality rates (log percent survival=$-Zd + Y$) for OTC ($-Z=0.0013$, $r^2=0.92$), alizarin ($-Z=0.0014$, $r^2=0.80$), and wire ($-Z=0.0016$, $r^2=0.78$) marking were significantly less than those for plastic ($-Z=0.0023$, $r^2=0.90$), red ($-Z=0.0025$, $r^2=0.96$), and green treatments ($-Z=0.0033$, $r^2=0.92$; Fig.1). Survival curves showed similar patterns with higher mortality in the first 40 days; thereafter mortality was reduced (Fig. 1).

Percent mark retention of alizarin, OTC, and wire tags were significantly greater than those of other treatments. The highest mark retention was observed for OTC- and alizarin-marked fish (100%; Table 1). Wire-marked fish also showed high retention rates (85–100 %). Red-, green-, and plastic-marked fish showed significant declines in tag retention over the 68 days (Table 1).

Mean SL and weight showed no significant difference among treatments on day 1, 25, 48, and 68 (Tables 2 and 3). Growth rates were similar among all treatments: 1.0–1.1 mm SL/d and 0.5–0.6 g wet wt/d (Tables 2 and 3).

Discussion

Wire tags provided the best overall performance of the marking methods tested over this two-month

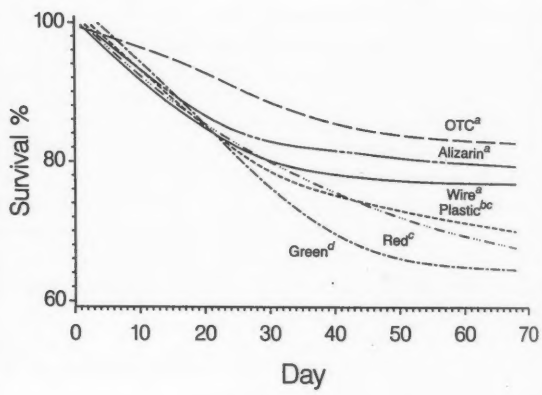

Figure 1

Percent survival of age-0 red drum, *Sciaenops ocellatus*, marked by six methods. Treatments with different letters were significantly different ($P<0.05$).

Table 1

Percent mark retention among sample days of age-0 red drum, *Sciaenops ocellatus*, marked by one of six methods: wire = coded wire microtag; plastic = external plastic tag; red = red fluorescent paint; green = green fluorescent paint; OTC = oxytetracycline dihydrate; and Ali = alizarin complexone. Numbers in parenthesis are sample sizes. Treatments with different letters are significantly different ($P<0.05$).

	Marking method					
Day	OTC[a]	Ali[a]	Wire[a]	Red[b]	Plastic[b]	Green[b]
25[a]	100.0(21)	100.0(20)	90.0(20)	80.5(82)	78.2(78)	42.3(78)
48[ab]	100.0(20)	100.0(20)	100.0(20)	55.8(52)	49.1(53)	39.1(46)
68[c]	100.0(19)	100.0(21)	85.0(20)	14.8(27)	24.1(29)	16.0(25)

study. Wire-marked fish showed low mortality and high tag retention compared with those marked by the plastic and paint methods. Also, individual fish are identifiable with wires which can be more useful than batch marking with OTC and alizarin. Of particular interest were the high tag-retention rates of wires (85–100 %). In past studies of wire tags injected in the cheek muscle of age-0 *S. ocellatus*, considerable tag loss was shown over the first 114 d: 67.3% tag retention after 24 h, 47% from 2 to 23 d, and 45%

Table 2

Mean standard lengths and growth rates of age-0 red drum, *Sciaenops ocellatus*, marked by one of six methods: wire = coded wire microtag; plastic = external plastic tag; red = red fluorescent paint; green = green fluorescent paint; OTC = oxytetracycline dihydrate; and Ali = alizarin complexone. Treatments were not significantly different ($P<0.05$). SL=standard length, SE=standard error, n=sample size.

		Mark method					
Day	Measure	Wire	Plastic	Red	Green	OTC	Ali
1	SL (mm)	65.9	66.9	66.8	67.1	68.9	67.6
	SE	0.8	0.9	0.9	0.9	0.9	0.7
	n	100	100	100	100	100	114
25	SL (mm)	90.5	91.6	88.8	90.5	90.7	91.6
	SE	1.0	1.1	1.1	1.1	1.1	1.0
	n	80	78	82	78	88	95
48	SL (mm)	116.7	117.9	112.5	118.1	117.5	114.0
	SE	1.3	1.7	2.0	1.6	1.6	1.4
	n	57	53	52	46	64	71
68	SL (mm)	131.1	134.7	134.4	134.3	128.7	129.1
	SE	2.2	2.9	2.0	2.3	1.8	2.1
	n	37	29	27	25	44	50
	Growth (mm/d)	1.1	1.1	1.1	1.1	1.0	1.0
	r^2	0.86	0.83	0.83	0.85	0.80	0.83

Table 3

Mean weights and standard error (SE) of age-0 red drum, *Sciaenops ocellatus*, marked by one of six methods: wire = coded wire microtag; plastic = external plastic tag; red = red fluorescent paint; green = green fluorescent paint; OTC = oxytetracycline dihydrate; and Ali = alizarin complexone. Treatments were not significantly different ($P<0.05$). WT=weight, n=sample sizes.

		Mark method					
Day	Measure	Wire	Plastic	Red	Green	OTC	Ali
1	WT (g)	4.9	5.2	5.2	5.2	5.7	5.2
	SE	0.2	0.2	0.2	0.2	0.2	0.2
	n	100	100	100	100	100	114
25	WT (g)	12.9	13.5	14.0	13.5	13.4	13.1
	SE	0.4	0.5	1.1	0.5	0.5	0.4
	n	80	78	82	78	88	95
48	WT (g)	30.1	29.5	27.9	28.2	27.8	27.2
	SE	1.1	1.3	1.4	1.1	1.1	1.0
	n	57	53	52	46	64	71
68	WT (g)	41.9	44.7	45.1	42.2	37.6	38.1
	SE	2.1	2.8	2.2	2.5	1.7	2.0
	n	37	29	27	25	44	50
	Growth (g/d)	0.6	0.6	0.6	0.6	0.5	0.5
	r^2	0.80	0.77	0.69	0.80	0.74	0.72

from 24 to 114 d (Bumguardner et al., 1992). A similar wire tag loss rate was observed in striped bass tagged horizontally in the cheek muscle (22.2–30.7% retention over the first 70 d; Dunning et al., 1990). However, Dunning et al., (1990) also reported that wire retention rates substantially increased when stripped bass were tagged in the snout (63–98.5%), nape (93.8–99.3%), or vertically in the cheek (82.7–87.0%) over the first 70 days. Also, Klar and Parker (1986) showed 99% tag retention after 90 days if wires were injected into the epaxial muscle of striped bass. Our results agree with the higher tag retention rates reported by Dunning et al., (1990) and Klar and Parker (1986); there was little indication of tag loss in the first days after marking. We suggest that higher wire retention resulted from mark location, because current wires were injected deep (4–5 mm) into the epaxial muscle and had little chance of expulsion.

Although wire tags showed the best overall performance compared with other tags, the method was the most labor intensive of all methods, because of the required dissection, removal, and reading of wires. If individual growth rates are needed and both personnel and budget are limiting, plastic tags may be useful. Plastic tags can be read directly without harm to the fish, but they also showed significant tag loss compared with other methods and should be limited to short experiments of no more than 25 days.

Paint marking methods showed greater mortalities and lower retention times compared with other methods but are useful in situations where fish can only be held for short periods (1–2 h, see Weinstien et al., 1984). However, paint marking methods should also be limited to short-term experiments because of significant mark loss after 25 days.

If fish movements or survival are the objectives and fish can be held for long (≈15 h) marking periods, then OTC or alizarin may be the best marking methods. Both methods showed higher tag retention (100%) and lower mortality rates compared with plastic minitags or paint methods. Other studies have shown long-term retention for these chemicals: for example, OTC marks in chum salmon, *Oncorhynchus keta* (Bilton, 1986) and alizarin marks in *P. major* (Tsukamoto et al., 1989) were both detected two years after marking. Another advantage of OTC and alizarin staining was that handling was minimal and probably caused the least amount of stress among all the marking methods, as reflected in the lower mortality rates. Therefore, these fluorescent stains would be most suitable for long-term studies where individual growth rates are not needed and for batch marking large numbers of fish, for example prior to release of hatchery reared fish (Tsukamoto et al., 1989; Secor et al., 1991a).

One difference in the present study was the use of oxytetracycline-dihydrate instead of oxytetracycline-hydrochloric acid (HCL), as used in other studies. (Hettler, 1984; Tsukamoto and Shima, 1990; Secor et al., 1991a). The dihydrate form of OTC was advantageous in that it did not cause a reduction in pH as observed with the HCL form. This difference may account for the low mortality observed after 15 hours in the OTC bath (i.e. no fish died during the 15-h marking period). One advantage of the alizarin stain over the OTC marker was the ease in detecting the fluorescent marks. When whole otoliths were examined only 2 of 91 alizarin otoliths needed further cutting and polishing to detect the stain, whereas 23 of 83 OTC otoliths needed sectioning before OTC marks were visible.

As indicated by growth rates, fish acclimated well to the closed seawater system. Although we did not have a control group of fish, growth rates in the present study (1.0–1.1 mm SL/d) were similar to or greater than previous studies of unmarked *S. ocellatus* of similar sizes and temperatures as the current study: 0.8 mm/d from Tampa Bay (Peters and McMichael, 1987), 0.8 mm/d from Charleston Harbor (Daniel, 1988), and 1.0 mm/d for reared fish in Texas (Colura et al., 1990). Also, we were not attempting to compare growth rates of marked fish with those of wild populations or those of unmarked laboratory fish but rather to determine the most useful tag of the methods tested.

Thus, we recommend wire tags when individual marks are needed because of lower mortality and higher mark retention compared with those from plastic minitags. We recommend alizarin when batch-marking methods are needed and individual growth rates are not critical, because of lower mortality and higher retention compared with those from paint methods and because of ease of detecting mark compared with OTC marking.

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Stranding and mortality of humpback whales, *Megaptera novaeangliae*, in the mid-Atlantic and southeast United States, 1985–1992

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Marine mammal strandings are a result of, or result in, mortality that may be attributed to natural or anthropogenic factors. As such, stranding data can provide insight on spatial distribution, seasonal movements, and mortality factors pertaining to marine mammal populations (Woodhouse, 1991; Mead[1]).

The general distribution and migratory movements of humpback whales, *Megaptera novaeangliae*, in the western North Atlantic are well known from numerous studies based on the identification of individual animals and on other techniques. Humpbacks feed in high latitude areas during the summer months, including waters of the Gulf of Maine, eastern Canada, West Greenland, and Iceland (Hain et al., 1982; Martin et al., 1984; Perkins et al., 1984; Katona and Beard 1990). In the winter, whales from all populations migrate to breeding grounds in the West Indies (Balcomb and Nichols, 1982; Mattila and Clapham, 1989; Mattila et al., 1989; Katona and Beard 1990). Between these migratory end points, little is known of the distribution of the species. In recent years, however, there has been an apparent increase in the frequency of sightings of humpback whales off the mid-Atlantic coast of the United States (Swingle et al., 1993). Furthermore, a considerable number of strandings have been documented along the mid-Atlantic and southeast coasts, many in midwinter, a time when the majority of humpbacks are thought to be located in tropical waters. In this paper, we analyze data from these strandings, discuss implications regarding distribution and possible spatial segregation by age class, and examine apparent causes of mortality.

Methods

Study area and period

The study covers the coastal area of eastern North America extending from New Jersey(40°28'5N, 74°00'0W) to southern Florida (25°12'N, 80°13'W), consisting of 2,319 km of coastline (Fig. 1). The eight-year period from 1 January 1985 through 31 December 1992 was investigated. Stranding data were obtained from the United States Museum of Natural History, Smithsonian Institution's Marine Mammal Events Program (MMEP). This information was confirmed and augmented by comparison with data from stranding response personnel involved with the Northeast and Southeast Regional Stranding Networks and with data published in newspaper reports. Organizations involved in the regional stranding networks operate under a permit issued by the National Marine Fisheries Service. The names and organizations of investigators responding to specific stranding events are on file.

Analyses

The following data were recorded for each stranding: date, location, sex, body length, and the presence or absence of body markings that may indicate a possible anthropogenic cause of mortality (e.g. ship strike or fishery interaction).

Stranding incidents among states were compared by using a ratio of the number of strandings in the state to

[1] Mead, J. G. 1979. An analysis of cetacean strandings along the eastern coast of the United States. *In* J. R. Geraci and D. J. St. Aubin (eds.), Biology of marine mammals: insights through strandings, p. 54–68. Report to U.S. Marine Mammal Comm. Contract MM7AC020. U.S. Dep. of Commer., Natl. Tech. Info. Serv. PB-293 890.

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the length of coastline along the state. This is referred to as the stranding incidence ratio (SIR). Length of coastline was calculated from Ringold and Clark (1980).

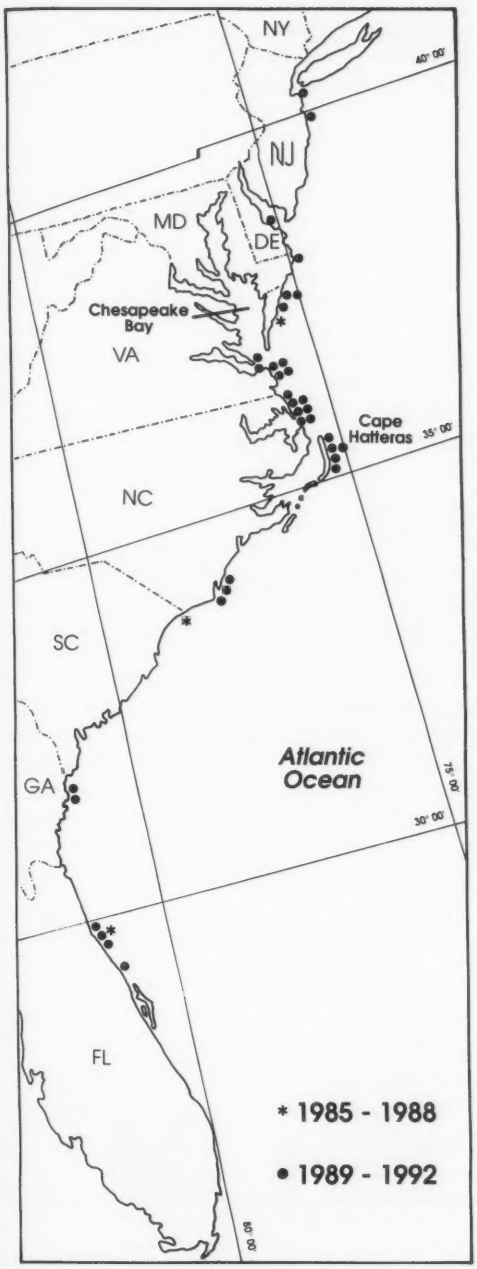

Figure 1

Locations of humpback whale, *Megaptera novaeangliae*, strandings from New Jersey to southern Florida, 1985 through 1992.

Reproductive class was inferred from body-length data. Animals of less than 8 m in length were considered to be dependent, nursing calves (Nishiwaki, 1959; Rice, 1963). We considered newly independent calves to be animals greater than 8.0 m but less than 9.9 m (calculated from Katona et al., 1983[2]). Males between 9.9 m and 11.6 m and females between 9.9 m and 12.0 m were considered sexually immature but not newly independent. Animals greater than 11.6 m (males) and 12.0 m (females) were considered sexually mature (Nishiwaki, 1959; Rice, 1963).

The Mann-Whitney *U*-test (Sokal and Rohlf, 1981) was used to test for differences between the number of strandings that occurred in the period 1985–88 versus 1989–92. Time periods were chosen to coincide with reported changes in observations of live animals in the same region (Swingle et al., 1993). The hypothesis that strandings occurred randomly throughout the study area was tested by chi-square analysis in a 2×2 contingency table (Sokal and Rohlf, 1981). The hypothesis that stranding events were not influenced by season was tested by chi-square analysis. Seasons were winter (January–March), spring (April–June), summer (July–September) and fall (October–December). Seasonal groupings were constructed so that the winter season would approximately coincide with the period of peak humpback occupancy of the breeding grounds, as reported by Mattila and Clapham (1989). The hypothesis that stranding occurrence was not influenced by sex was tested by chi-square analysis in a 2×2 contingency table.

Factors relating to mortality were taken from the written reports of on-site stranding response personnel from the Northeast and Southeast Regional Stranding Networks or, when not available, from the synthesis of such reports contained in the MMEP. The experience of stranding network response personnel is variable, and factors contributing to death or interpretation of bodily injury can be subject to debate. If on-site investigators recorded references to rope marks, propeller marks, broken bones, large gashes, etc., or directly suggested ship strike or entanglement as a potential cause of death, we attributed the death to possible anthropogenic causes. All mortality not suggesting anthropogenic trauma were grouped into a "natural" mortality category. This included animals that were euthanized but showed no other indications of human interaction. If a necropsy was conducted and no mention was made of body trauma, we assumed natural mortality. Carcasses that were reported to be in advanced stages of decomposition were eliminated from consideration.

[2] Calculated as length at birth, 4.5 m; growth rate, 45 cm per month; 12 month growth period = 9.9 m.

Results

A total of 38 stranded humpback whales were recorded between 1 January 1985 and 31 December 1992 (Table 1). One animal (4/14/85) was not included in the analyses because body condition ("mummification") indicated death or stranding, or both, occurred prior to the study period. The number of strandings by year was as follows: 0 in 1985, 2 in 1986, 0 in 1987, 1 in 1988, 3 in 1989, 8 in 1990, 7 in 1991, and 16 in 1992. Significantly more animals stranded during the period 1989 to 1992 (n=34), than from 1985 to 1988 (n=3) (Mann-Whitney U: Z=–2.32, P=0.02). Of the strandings recorded in our database, 92% (34/37) occurred after January 1989.

Significantly more strandings occurred along 170 km of coastline between Chesapeake Bay, Virginia, and Cape Hatteras, North Carolina (χ^2=70.67, df=1, P<0.01), than occurred in the rest of the study area. In this region, which represents 7.3% (170 km/2,319 km) of the coastline within the study area, 43% (16/37) of all strandings occurred. A second cluster of strandings occurred along the coast of northern Florida; however, this grouping was not found to be significant (χ^2=5.98, df=1, P=0.25). The region, which represents 4.7% (110 km/2319 km) of the study area's coastline, contained 13.5% (5/37) of all strandings.

The number of strandings per state was highly variable (Table 2). Numerically, the highest number of strandings occurred in North Carolina (n=15), but the incidence of strandings (strandings per kilometer of coastline) was greatest in Virginia (SIR=0.055, n=10), followed by North Carolina (SIR=0.031). South Carolina had the lowest incidence of strandings (SIR=0.003, n=1). The stranding incidence ratio for the entire study area was 0.016. All states recorded at least one stranding.

There were no significant differences in stranding occurrence by season (χ^2=4.22, df=3, P=0.24) (Fig. 2). However, only 8% (3/37) of all strandings occurred during the summer (July-September). Strandings occurred with the greatest frequency in April (n=6) followed by February, March, and October (n=5 each), and least in July and August (n=0 each). In 1992 (the most recent year of the study), strandings were spread over a greater number of months than any of the seven previous years.

Data on body length were available for 25 animals. Body length indicated all animals were sexually immature but none were dependent calves. Sixty-eight percent (17/25) of the animals were considered newly independent calves. Information on gender was available for 26 animals. Fifty percent (13/26) were female and 50% (13/26) were male.

Mortality

Of the 37 animals, an advanced stage of decomposition eliminated 13 from analysis for potential cause of death. Four additional animals were insufficiently examined or information was inadequately reported to determine a cause of death or the presence or absence of injury or scars. Of the 20 remaining animals, 30% (6/20) had major injuries potentially attributable to a ship strike and 25% (5/20) had injuries consistent with possible entanglement in fishing gear. One animal exhibited scars consistent with past entanglement or ship strike, or both, and was emaciated at the time of stranding. Thus, up to 60% (12/20) of the sufficiently inspected animals showed signs that anthropogenic factors may have contributed to or been directly responsible for their death. However, the possibility that some animals sustained body trauma after death can not be ruled out. Unfortunately, few animals were sufficiently necropsied to establish an unequivocal cause of death.

Discussion

These results suggest that stranding of humpback whales along the mid-Atlantic and southeast coastal areas of the United States has increased. All stranded animals were sexually immature and males and females stranded with equal frequency. However, natural mortality may show a gender bias that has been obscured by the high number of deaths potentially due to anthropogenic factors. Strandings occurred throughout the fall, winter, and spring seasons, but few strandings occurred during the summer months.

There are several possible explanations for the apparent increase in strandings, including changes

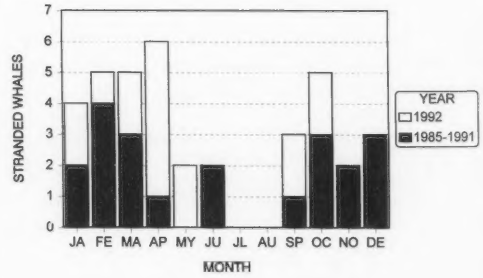

Figure 2

Humpback whale, *Megaptera novaeangliae*, strandings by month, 1985 through 1992.

Table 1

Humpback whale, *Megaptera novaeangliae*, strandings, New Jersey to south Florida, 1985–1992. unk = unknown; est = estimated.

Date	Location	Sex	Length	Necropsy	Carcass analyses	Potential cause of death
14 Apr 85	Carolina Beach, NC 34°02'--" N 078°53'--" W	unk	unk	no	old carcass (mummy or skeleton) not included in analyses	unknown
15 Feb 86	Cobb Island, VA 37°2-'--" N 075°4-'--" W	F	10.8 m	partial	fresh, no obvious sign of external trauma or disease	natural
07 Mar 86	N. Myrtle Beach, SC 33°48'--" N 078°44'--" W	F	11.7 m	yes	live stranding; euthanized	natural
08 Dec 88	St. Johns, FL 29°54'--" N 081°20'--" W (est)	unk	7.8 m (est)	no	advanced decomposition	unknown
14 Jan 89	St. Augustine, FL 29°55'3-" N 081°17'3" W	F	7.6 m (est)	unknown	advanced decomposition	unknown
18 Sep 89	Monmouth Beach, NJ 40°19'55" N 073°57'17" W	unk	8.0 m (est)	no	entangled in gear, apparently anchored by gear to bottom	entanglement
18 Dec 89	Assateague Island, VA 37°50'--" N 075°20'--" W	F	8.7 m[1]	yes	live stranding, no external injuries noted	natural
27 Jan 90	New Smyrna Beach, FL 29°00'0-" N 080°52'2-" W	M	7.9 m	yes	advanced decomposition	unknown
05 Feb 90	Nags Head, NC 35°56'5-" N 075°36'5-" W	unk[2]	11.1 m	partial	broken jaw bone, head damaged[3]	ship strike
24 Feb 90	Corolla Beach, NC 36°15'--" N 075°46'--" W	unk	9.0 m (est)	unknown	fresh, insufficient information	unknown
24 Mar 90	Sanderling, NC 36°11'5-" N 075°45'2-" W	unk	7.6 m – 8 m (est)	no	advanced decomposition	unknown
01 Apr 90	Virginia Beach, VA 36°4-'--" N 075°5-'--" W	F	9.6 m	yes	fresh, net/line marks on tail stock, right half of fluke had line marks	entanglement
19 Jun 90[4]	Virginia Beach, VA 36°56'15" N 076°03'30" W	F	8.3 m	yes	fresh, no evidence of scars or injuries	natural
20 Jun 90	Virginia Beach, VA 36°45'15" N 075°56'30" W	F	8.2 m	yes	live stranding; euthanized, rope marks on flukes, emaciated	entanglement

Table 1 (continued)

Date	Location	Sex	Length	Necropsy	Carcass analyses	Potential cause of death
19 Nov 90	Norfolk, VA 36°56'00" N 076°11'30" W	M	9.5 m	no	various rope burns, abrasions on tail stock, rope scars on left flipper	entanglement
05 Feb 91	St. Johns, FL 29°59'06" N 081°18'48" W	M	9.4 m	partial	moderately decomposed, no external injuries noted	natural
02 Mar 91	Bald Head Island, NC 33°55'0-" N 077°56'4-" W	M	8.5 m	no	inaccessible	unknown
15 Oct 91	Kill Devil Hills, NC 36°01'--" N 075°39'--" W	unk[5]	9.3 m[6]	partial	no external injuries noted	natural
25 Oct 91	Nags Head, NC 35°56'5-" N 075°37'0-"	M	9.1 m (est)	no	no external injuries noted	natural
27 Oct 91	Bodie Island, NC 35°46'0-" N 075°29'1-" W	unk	10.0 m	no	advanced decomposition	unknown
08 Nov 91	Island Beach State Park, NJ 39°50'00" N 074°05'12" W	M	9.0 m	yes	four propeller cuts, one through the occipital condyle, were cause of death	vessel strike
25 Dec 91	Carolina Beach, NC 34°01'--" N 077°54'--" W	F	9.9 m	no	insufficient information	unknown
03 Jan 92	Salvo, NC 35°20'9-" N 075°21'8-" W	M	10.4 m	no	no external injuries noted	natural
30 Jan 92	Oregon Inlet, NC 35°46'5-" N 075°31'9-" W	unk	unk	no	inaccessible	unknown
14 Feb 92	Virginia Beach, VA 37°01'--" N 076°07'--" W (est)	M	8.5 m[1]	yes	left eye socket and left mandible fractured, signs of healing from injuries at point of fractures	vessel strike
10 Mar 92	Avon, NC 35°20'--" N 075°21'--" W	F	10.7 m	partial	left fluke "scalloped" possibly due to ship strike or entanglement, evidence of healed rope/net scars on caudal peduncle	past entanglement or ship strike
19 Mar 92	North Core Banks, NC 35°01'1-" N 076°06'0-" W	M	11.0 m	no	advanced decomposition	unknown

Table 1 (continued)

Date	Location	Sex	Length	Necropsy	Carcass analyses	Potential cause of death
14 Apr 92	St. Johns, FL 29°45'--" N 081°10'--" W (est)	unk	8.6 m existing length	no	advanced decomposition	unknown
16 Apr 92	Assateague Island, MD 38°12'--" N 075°08'--" W	F	8.9 m	yes	no external trauma, but skull disarticulated, blunt trauma to left side	vessel strike
18 Apr 92	Southport, NC 33°42'8-" N 77°55'4-" W	M	9.5 m	no	advanced decomposition	unknown
22 Apr 92	Hatteras, NC 35°11'4-" N 075°46'3-" W	F	8.9 m	yes	no external trauma, but extensive skeletal damage, "probably struck by boat"	vessel strike
30 Apr 92	Nags Head, NC 35°22'--" N 075°29'--" W	unk	9.2 m (est)	no	advanced decomposition inaccessible	unknown
16 May 92	Ossabaw Island, GA 31°45'7-" N 081°05'0-" W	M	> 7.2 m	no	advanced decomposition	unknown
17 May 92	St. Catherines Island, GA 31°38'2-" N 081°08'2-" W	unk	unk	partial	advanced decomposition	unknown
22 Sep 92	Prime Hook National Wildlife Refuge, DE 38°55'--" N 075°05'--" W	F	8.3 m (est)	yes	advanced decomposition	unknown
28 Sep 92	Assateague Island, VA 37°53'--" N 075°22'--" W	M	8.9 m (est)-part of head buried	yes	advanced decomposition	unknown
09 Oct 92	Metompkin Island, VA 37°46'--" N 075°32'--" W	F	8.7 m	yes	"probably boat strike," 3 areas of hemorrhage noted	vessel strike
22 Oct 92	Virginia Beach, VA 36°46'15" N 075°57'02" W	M	9.1 m	yes	"obvious entaglement scars" on leading edge of fluke and around caudal peduncle	entanglement

[1] Animal towed prior to measurement, therefore measured length may be greater than actual length.

[2] Discrepancy in reported gender. Original stranding report stated female. MMEP reported male.

[3] Discrepancy in reported body condition. Original stranding report stated broken jaw bone and head damage. MMEP had no report of body condition.

[4] Discrepancy in reported date. Original stranding report stated 19 June 1990. MMEP reported 19 May 1990.

[5] Discrepancy in reported gender. Original stranding report stated female. MMEP reported as unknown.

[6] Discrepancy in reported body length. Original stranding report stated 9.3 m. MMEP reported an estimated length of 660 cm.

Table 2

Humpback whale, *Megaptera novaeangliae*, strandings by state; 1985 through 1992.

State	Number of strandings	Kilometers of coastline	SIR: Number of strandings / km of coastline
Virginia	10	180.6	0.055
North Carolina	15	485.5	0.031
Delaware	1	45.2	0.022
Maryland	1	50.0	0.020
Georgia	2	161.3	0.012
New Jersey	2	209.7	0.010
Florida	5	935.5	0.005
South Carolina	1	301.6	0.003

in observer effort, mortality factors, and whale distribution. That increased observer effort could account for the increase seems unlikely. The size of stranded humpback whales and both the public and media interest in such events results in few carcasses escaping notice. Additionally, strandings of finback whales, *Balaenoptera physalus*, over the same time period have remained relatively constant (1985 to 1988, n=10; 1989 to 1992, n=9)) (calculated from MMEP, Smithsonian Institution). An increase for this large baleen species might also be expected if the reported humpback change were due solely to increased observer effort.

If the reported increase in strandings is not an artifact of observer effort, it may be due to an increase in factors resulting in mortality, an increase in the number of animals inhabiting the study area, or both. While the tonnage of cargo moving through Atlantic ports in 1989 showed a 9% increase over the mean of the previous four years (calculated from Anon., 1991), the number of vessels using the Chesapeake Bay area, and probably the rest of the Atlantic coast, has decreased because ships capable of carrying greater tonnage are being used (Pringer[3]). While a decline in vessel traffic may result in a decreased risk to whales, it is possible that these larger, faster, deeper draft vessels pose a greater danger than the slower, shallower draft vessels of the past. In addition to commercial shipping, some areas, such as near Chesapeake Bay and northern Florida, are subject to substantial use by military vessels. However, data pertaining to trends in military vessel traffic were not available.

Evidence also indicates that as much as 25% of the reported mortality may be attributable to interaction with commercial fishing activity, such as gill netting. North Carolina's coastal sink gillnet fishery expanded dramatically during the 1980's (Ross[4]). South Carolina, the state with the lowest SIR, banned the commercial use of gill nets in 1987 (with the exception of a tended shad net fishery) (Moran[5]). However, fishing effort in the entire study area is inadequately monitored to determine trends (Read, in press; Bisack[6]).

While changes in shipping and commercial fishing activity may represent increased hazards to animals inhabiting the study area, they seem inadequate to account for the dramatic change in stranding levels reported. Each of these hazards existed prior to 1989, the period when strandings began to increase. The most likely explanation for the reported increase in mortality appears to be increased use of this area by juvenile humpback whales that are then exposed to such hazards.

Although few standardized marine mammal surveys consistently cover the study area, anecdotal and published observations point to a recent increase in live sightings of humpback whales in coastal waters of Florida and Georgia (Kraus[7]), North Carolina (Barrington[8]), Virginia (Swingle et al., 1993), and Maryland (Driscoll[9]). Although reliable estimation of the length of free-swimming whales is difficult, there is general agreement among observers that most, if not all, of the animals frequenting the area are small.

Changes in humpback whale distribution in relation to changes in prey composition and abundance have been demonstrated elsewhere (Payne et al., 1986; Piatt et al., 1989; Payne et al., 1990), and such a prey shift may account for or be an important fac-

[3] Pringer, Captain M. Association of Maryland Pilots, Baltimore, MD 21228. Personal commun., January 1993.

[4] Ross, J. L. 1989. Assessment of the sink net fishery along North Carolina's Outer Banks, fall 1982 through spring 1987, with notes on other coastal gill net fisheries. Special Sci. Rep. 50, North Carolina Dep. of Environ., Health and Nat. Resour., Moorehead City, NC, 54 p.

[5] Moran, J. South Carolina Wildlife and Marine Resource Department, Charleston, SC 29422. Personal commun., September 1993.

[6] Bisack, K. 1992. Sink gill net fishing activity in the North Atlantic as reflected in the NEFSC weightout database: 1982–1991. U.S Dep. Commer., NOAA, Natl. Mar. Fish.Serv. Northeast Fish. Sci Cent., Woods Hole, MA 02543. Unpubl. manuscr., 4 p.

[7] Kraus, S. New England Aquarium, Boston, MA 02110. Personal commun., March 1993.

[8] Barrington, P. North Carolina Aquarium, Fort Fisher, Kuri Beach, NC 28449. Personal commun., April, 1993.

[9] Driscoll, C. NMFS, Office of Protected Resources, Silver Spring, MD 20910. Personal commun., March 1993.

tor in the change in whale distribution suggested here. While data on changes in prey distribution were not available, the first observations of winter feeding humpbacks were documented in the nearshore waters of Maryland (deGroot[10]) and Virginia (Swingle et al., 1993), during the winters of 1991 and 1992.

An additional possibility is that the humpback whale population in the western North Atlantic may be increasing and expanding its range such that habitats historically occupied are being recolonized. Several authors (Katona and Beard, 1990; Sigurjonsson and Gunnlaugsson, 1990) have reported numerical increases for this population, although this may be due to increased effort resulting in more accurate estimates of abundance.

Humpback whales may have always been present during winter in offshore waters of the study area, but a shift in prey abundance or distribution, or both, may have brought them into areas where death would result in stranding, rather than have caused them to be lost at sea. However, offshore concentrations were not detected during 1978–82 aerial surveys (CeTAP[11]) or during 1980–88 ship board surveys (Payne et al.[12]).

While juvenile whales can be expected to exhibit higher mortality than adults (Sumich and Harvey, 1986; Kraus, 1990a), the absence of adult animals from the stranding record may provide support for the suggestions of Swingle et al. (1993) that winter or migratory segregation, or both, is occurring. Foraging opportunities on the breeding grounds are rare (Dawbin, 1966; Baraff et al., 1991), and it may be adaptive for some juvenile animals to remain and feed in mid-latitude areas, rather than to migrate with adults. If occupying the breeding grounds is the preferred behavior, individuals remaining in higher latitude areas may be those that failed to obtain sufficient resources during the feeding season. Such nutritionally stressed animals may be more susceptible to all forms of mortality, natural or anthropogenic. Nutritionally stressed juveniles and newly weaned calves in particular may be vulnerable to the effects of the parasitic nematode *Crassicauda boopis* (Lambertsen, 1992).

If winter foraging opportunities are sufficient, juveniles may delay their return to traditional feeding areas and may eventually occupy new habitat. This may be one mechanism by which a species establishes itself in new areas or reoccupies historic sites. This process may be reflected in the stranding record. There seems to be a progressive trend not only for an increased number of strandings but for strandings to take place in a greater variety of months.

A high percentage of animals exhibited signs that anthropogenic interactions could be implicated in their death. However, there are reasons to believe that mortality estimates based on available stranding data could under- or overestimate the impact of human interaction. For example, stranded animals on 16 and 22 April 1992 exhibited no external signs of trauma. However, necropsies indicated internal injuries consistent with a ship strike (McLellan[13]; Thayer[14]), suggesting that such injuries could have escaped notice during more cursory examinations. The lack of external body trauma on animals which thorough necropsy revealed to have been killed by a ship strike has also been noted for the northern right whale, *Eubalaena glacialis* (Kraus[15]).

Alternatively, references to rope or net marks did not always specify whether such marks were of recent origin or due to past entanglement from which the animal escaped. Baleen whale entanglement does not always lead to immediate mortality (Kraus, 1990a); however, the effect of escaped entanglement on long-term survivorship or reproductive success, or both, is unknown. If rope or net marks noted in the stranding reports were of past origin, they may have been independent of the animal's death or the animal may have succumbed to the long-term effects of past entanglement. Reduced foraging efficiency during the entanglement period may be a factor influencing animals to engage in winter feeding behavior, such as that observed in the study area by Swingle et al. (1993).

The apparent high rate of interaction with commercial fishing and shipping, may be due, in part, to the age class inhabiting the area. Juvenile animals, and newly independent calves in particular, may be more susceptible to ship strikes or fishing gear entanglements, or both, owing to a lack of experience with either factor (Lien, in press). Commercial shipping and military traffic to and from the Chesapeake Bay passes by much of the area where strandings

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occur most frequently (Virginia and North Carolina), often in water depths of less than 20 m. In Florida, the concentration of strandings occurs in the vicinity of active commercial and military shipping and where ship strikes have been reported to represent a hazard to northern right whales (Kraus and Kenney, 1991).

Entanglement in commercial fishing gear has been the most frequently identified anthropogenic cause of injury and death in humpback whales; gillnet-type gear most often was implicated (O'Hara et al., 1986). Coastal gillnet fisheries exist in the study area on a year-round basis, but effort may peak in late winter/spring (NMFS, 1992; Swingle et al., 1993; Brooks[16]). Over 2,200 gillnet licenses have been issued for the mid-Atlantic coastal region. However, fishermen may hold more than one permit and some coastal fisheries do not require permits (NMFS, 1992). In the study area, coastal gill nets and whales concurrently occupy waters of less than 15 m in depth (observed by RAA and DPG), and whales have been observed trailing such gear (Swingle[17]). The association of young, inexperienced whales with gill nets in shallow waters may increase the potential for entanglement incidents. Since entanglement mortality is inversely related to body size (Lien et al., 1989; Kraus 1990b), juvenile humpbacks may be more susceptible to fatalities.

Data contained in this paper suggest that mid-Atlantic and southeast coastal areas of the United States are becoming increasingly important habitat for juvenile humpback whales and that anthropogenic factors may negatively impact these animals. However, there are a number of factors that suggest caution should be used in interpretation of these data. The site of stranding is not necessarily the site of death, as the body of a large whale can be carried considerable distances by wind and currents before beaching occurs. Cause of death in the stranded animals was rarely determined with certainty and in most cases was inferred from observations of the presence or absence of surface body trauma, not from thorough necropsy by experienced individuals. A greater emphasis should be placed on complete necropsies of stranded animals to determine not only the immediate cause of death but also whether there is an underlying factor in the fatality. This would allow a more reliable investigation into mortality and provide greater ability to evaluate and alleviate the impact of anthropogenic interactions. This is particularly important for an endangered species, such as the humpback whale.

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National Marine Fisheries Service, NOAA

The Publications Advisory Committee of the National Marine Fisheries Service is pleased to announce the awards for best publications authored by NMFS scientists and published in the Fishery Bulletin for Volume 91 and in the Marine Fisheries Review for Volume 54. Eligible papers are nominated by the Fisheries Science Centers and Regional Offices and are judged by the NMFS Editorial Board. Only articles that significantly contribute to the understanding and knowledge of NMFS-related studies are eligible. We offer congratulations to the following authors for their outstanding efforts.

Fishery Bulletin, Volume 91

Outstanding Publication

Ambrose Jearld Jr., Sherry L. Sass, and Melinda F. Davis

Early growth, behavior, and otolith development of the winter flounder *Pleuronectes americanus*. Fish. Bull. 91:65–75.

Ambrose Jearld is with Northeast Fisheries Science Center, Woods Hole, Massachusetts. Sherry Sass is with the Division of Marine Fisheries, Sandwich, Massachusetts. Melinda Davis is with Fort Valley State College, Fort Valley, Georgia.

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Detection of contaminant and climate effects on spawning success of three pelagic fish stocks off southern California: Northern anchovy *Engraulis mordax*, Pacific sardine *Sardinops sagax*, and chub mackerel *Scomber japonicus*. Fish. Bull. 91:310–327.

Michael Prager is with the Southeast Fisheries Science Center, Miami, Florida. Alec MacCall is with the Southwest Fisheries Science Center, Tiburon, California.

Marine Fisheries Review, Volume 54

Outstanding Publication

Thomas K. Wilderbuer, Gary E. Walters, and Richard G. Bakkala

Yellowfin sole, *Pleuronectes asper*, of the eastern Bering Sea: biological characteristics, history of exploitation, and management. Mar. Fish. Rev. 54(4):1–18.

Thomas Wilderbuer and Gary Walters are with the Alaska Fisheries Science Center, Seattle, Washington. Richard Bakkala is a retired fishery biologist who was formerly with the Alaska Fisheries Science Center, Seattle, Washington.

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Disease risks associated with importation of nonindigenous marine animals. Mar. Fish. Rev. 54(3):1–10.

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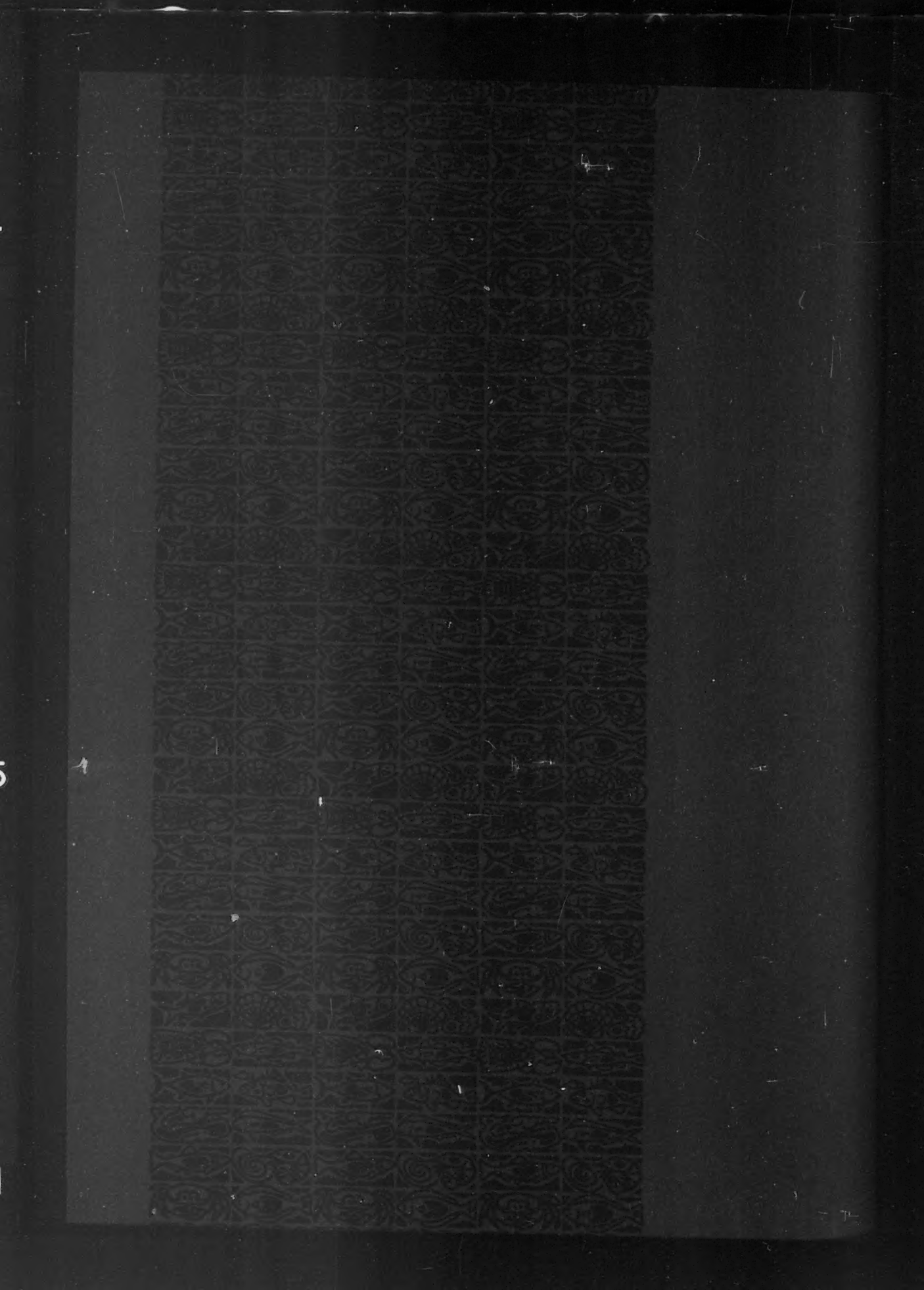
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